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A SOLUBLE SUBSTANCE IN CORNSTALKS THAT RETARDS GROWTH OF DIPLODIA ZEAE IN CULTURE [1]

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INTRODUCTION

As a rule, corn (*Zea mays* L.) plants are resistant to stalk rot, caused by *Diplodia zeae* (Schw.) Lév., during the period of their vegetative growth. Increase in susceptibility occurs after pollination. According to Koehler and Holbert (*14*),[3] the disease seldom reaches the stage where it becomes noticeable until the ears are in the milk stage or the kernels have dented. Thus differences in susceptibility among lines and among individuals within a line become evident as the plants near maturity.

The amount of stalk rot fluctuates from year to year. Weather and soil conditions affect host and parasite individually and their interaction in the course of disease development. As a consequence, field tests to determine the relative resistance of various lines must extend over a period of years.

It is recognized that resistance to disease may depend primarily on the anatomy or the physiology of the host plant or on various combinations of the two factors. In the case of corn, an erect, mechanically strong stalk and a large, well-anchored root system are desirable anatomical features from an agronomic point of view and tend to be interpreted as indicative of disease resistance.

With these conditions in mind, the present work was undertaken to study a phase of physiological resistance to stalk rot. The investigation sought evidence of the presence of and seasonal change in a substance in the expressed juice and ether extracts of stalks of yellow dent corn, resistant or susceptible to *Diplodia zeae*, that would affect the growth of the fungus in culture. It was presumed that the results of the laboratory tests would be relative and that, based on field material, they might be expected to vary from season to season; but it was thought that over a period of years trends might become evident that would correlate in some measure with the resistance and susceptibility of plants in the field.

MATERIAL AND METHODS

The corn plants used were grown on the University farms at Madison, Wis. At the beginning of the study, the susceptible inbred Ill. Lan and the more resistant Ill. R4 were the contrasting lines selected.

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[2] Grateful acknowledgment is made to Donald Bowman, P. E. Hoppe, Orme S. Kellett, and E. C. Stevenson for assistance in the field work, to W. J. Olson for help in making the extracts, to Eugene H. Herrling for the photography involved in the problem, and to Dr. J. G. Dickson for helpful suggestions during the preparation of the manuscript. In the cultivation of the field plots, limited use was made of assistance from the Federal Work Projects Administration, Natural Science Project of the University of Wisconsin.

[3] Italic numbers in parentheses refer to Literature Cited, p. 109.

Later, another inbred, Wis. 6, was used as the comparative standard for susceptibility to stalk rot. Other inbred lines tested at various times were Ill. L,[4] Ill. 90, Ill. Hy, Wis. 23, Wis. 26, Wis. R3, Ind. B2, Ohio 10, and Ohio 56. The hybrids used were the single crosses Ill. 90×Ill. K, Ill. R4×Ill. Hy, Ill. R126×Ill. Lan, Wis. R3×Wis. 22, Wis. 23×Wis. 24, Wis. 23×Ind. WF9, and Ind. WF9×Wis. 22.

Periodic collections of stalk tissue were made. The number of plants in each sample ranged from 2 to 15 in different years. The stalks were stripped of their leaves immediately after collection, and comparable portions of the stalk tissue between crown and ear node were used as samples. Freshly chopped portions were dried at 60° C. in an oven equipped with a fan. The dried material was enclosed in tin cans and stored at room temperature until needed. Later this material was ground in a Wiley mill, to pass a 2-mm. sieve, and extracted with ether. The excess of ether was distilled off, and the volume of the extract was adjusted so that each cubic centimeter represented 2 gm. of air-dry stalk meal. The largest number of extracts prepared in any one season was 84, in 1938. Samples of plants collected from the field were not replicated, because of insufficient time and space for the preparation of such large numbers of extracts.

The influence of the ether extracts of stalk tissue on the growth of *Diplodia zeae* in culture was determined by adding 4 to 10 cc. of the extract to 100 cc. of hot synthetic [5] or potato agar containing 2 percent of dextrose or sucrose. After the agar had cooled somewhat, the bottles were well shaken and the contents of each was poured into four 9-cm. Petri dishes. By the time the agar had solidified, no odor of ether remained. The addition of corresponding quantities of pure ether to the control plates did not affect the size of the colonies of the fungus. One colony was grown on each plate. At first, various types of inoculum were used; later, a single drop of spore suspension placed in the center of the plate was adopted as a standard procedure. The diameter of the colonies was measured after the plates had been stored for 3 to 7 days under ordinary laboratory conditions.[6] Temperature, humidity, and light were not controlled in the room, nor was the inoculum used in the different platings of uniform age. Hence, colonies in one plating might differ in size from colonies of the same age plated at another time. However, in any one series, the conditions are thought to have been uniform and the colonies comparable. During the course of the work, several strains of *D. zeae* were used without material difference in the results obtained. The same was true of the agars used. Because the fungus grew more slowly on synthetic agar it was preferred when large numbers of colonies were to be measured.

Freshly chopped stalk tissue was ground for juice extraction in a Nixtamal mill. The juice was expressed in a Carver press under

[4] P. E. Hoppe has grouped a number of lines used in this work, with regard to their general resistance or susceptibility to stalk rot in the field, as shown by premature death of the plants or by the extent of discoloration visible on the surface of the stalks after hypodermic inoculation with *Diplodia zeae* in 1 year or more. His classification is as follows: Very susceptible, Ill. Lan and Wis. 6; susceptible, Ill. R4, Wis. 23, and Wis. 23×Wis. 24; intermediate, Ohio 10, Ill. L, Ill. 90, and Wis. 23×Ind. WF9; resistant, Wis. 26, Wis. R3, and Ohio 56; very resistant, Ind. B2 (one season), Wis. R3 ×Wis. 22, and Ind. WF9×Wis. 22.

[5] Asparagin, 2 gm.; KH_2PO_4, 2 gm.; $MgSO_4$, 1.25 gm.; carbohydrate, 20 gm.; agar, 17.5 gm.; distilled water, 1,000 cc.

[6] In the discussion of results obtained, unless otherwise indicated, the terms "resistance" and "susceptibility" as applied to the host will refer to the retarding effect of the juices and extracts on the growth of *Diplodia zeae* in culture.

pressures of 6,000 to 8,000 pounds, filtered through a Büchner filter, and made sterile by passage through a Seitz filter. A part of the filtrate was used in hanging drops to test the effect of the juices on the germination and growth of the spores of *Diplodia zeae*. Agar was added to another portion of the juice to make a 1.7-percent agar medium, which was autoclaved and used for plate study of the growth of the fungus. In 1937, juice agar was prepared by adding agar to expressed juice that had been centrifuged for 5 minutes instead of being filtered. This method was satisfactory and had the advantage of greater speed of preparation. Autoclaving at 15 pounds' pressure for 30 minutes, or at 20 pounds for 15 minutes, did not destroy the growth-retarding principle in the juice. Quadruplicate plates were used, and each plate contained approximately 25 cc. of medium.

When periodic collections of healthy stalks were made, a few similar plants in each plot were inoculated with a spore suspension of *Diplodia zeae* introduced into the stalk by means of a hypodermic needle. These stalks, which were intended to indicate the relative susceptibility to stalk rot, were harvested at intervals and split, and the lesions were photographed.

EXPERIMENTAL STUDIES

INFLUENCE OF SUBSTANCES OBTAINED FROM CORNSTALKS ON GROWTH OF FUNGI

Ether Extracts from Normal Stalks

The study of the influence of ether extracts of stalk tissue on the growth of *Diplodia zeae* in culture was begun in a preliminary way in 1934 and was continued from 1935 through 1940. In all, 245 extracts were prepared and added to agar, and approximately 4,700 colonies were measured in as many plates. Relative growth of the fungus on any series of extracts varied so little in repeated platings that averages of few or many colonies seldom altered materially the trends shown (table 1). The inbreds chosen for test were selected

Table 1.—*Effect of 4-percent ether extracts of various corn lines on growth of Diplodia zeae, 1935*

Line and harvest date	Mean diameter of colonies on quadruplicate plates		Mean diameter for 8 colonies	Colonies in all platings	
	4-day-old potato-sucrose agar cultures	7-day-old synthetic-sucrose agar cultures		Total	Mean diameter
	Cm.	*Cm.*	*Cm.*	*Number*	*Cm.*
Ill. Lan:					
Aug. 28	5.6	5.2	5.4	36	5.6
Sept. 25	6.4	6.4	6.4	24	6.8
Oct. 9	8.4	8.9	8.7	36	8.8
Ill. 90 × Ill. K:					
Aug. 28	6.5	5.8	6.2	36	6.4
Sept. 25	6.8	7.3	7.1	20	7.1
Oct. 18	8.6	7.9	8.3	16	8.8
Ill. R4:					
Sept. 5	4.4	4.2	4.3	24	4.1
Sept. 25	5.2	5.2	5.2	28	4.4
Oct. 18	3.4	3.4	3.4	20	3.3

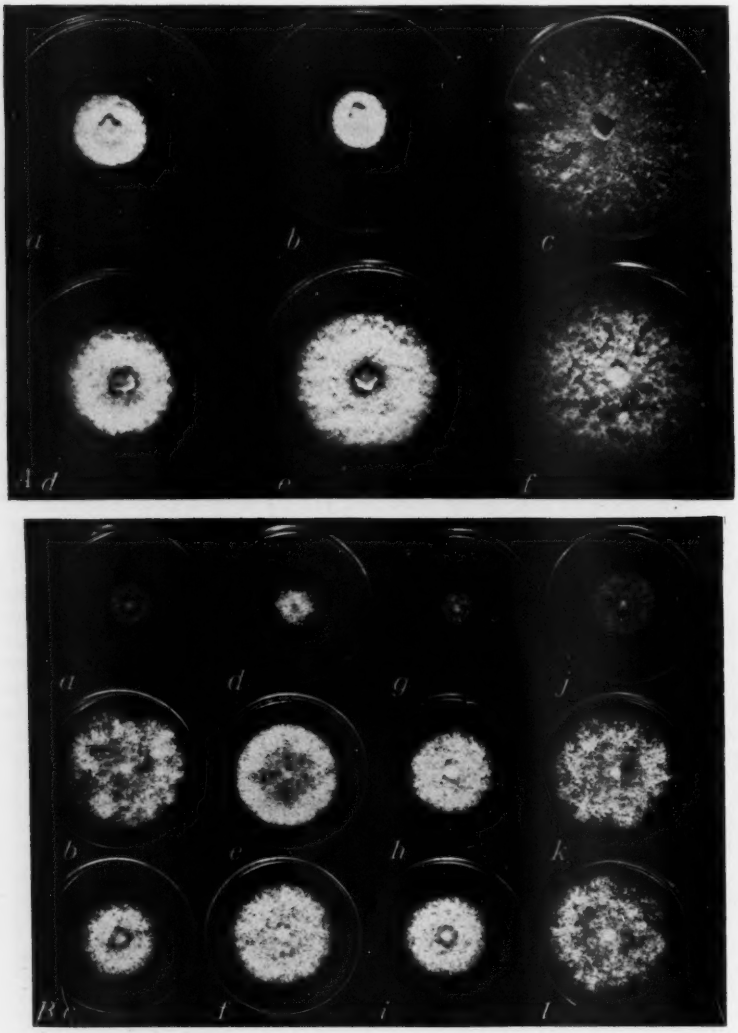

FIGURE 1.—Colonies of *Diplodia zeae* on (*A*) potato-dextrose agar and (*B*) synthetic agar, containing ether extracts from stalks of various inbreds collected on different dates. *A*, *a* and *b*, Extracts from inbred Ill. R4, collected September 5 and October 18, 1935, respectively; *c*, control (no extract added); *d–f*, extracts from inbred Ill. Lan, collected August 28, September 25, and October 9, 1935, respectively. *B*, Extracts from the following inbreds: *a–c*, Wis. 6; *d–f*, Wis. 26; *g–i*, Wis. 23; *j–l*, Wis. R3, each collected August 5 and 25 and September 9, 1938, respectively.

for a variety of characters. They showed a range in the number of days required to reach maturity, in the strength of their stalks and extent of their root systems, and in their resistance or susceptibility to stalk rot. Of the inbreds tested, only the 11 lines that had been used for 2 years or more or had entered into the hybrids have been included in this report together with 5 hybrids.

The results obtained indicate in general the presence in cornstalks, during the period of vegetative growth, of an ether-soluble substance that retards the spread of *Diplodia zeae* in culture (fig. 1). This substance tended to diminish for a time after pollination. Some lines consistently reached higher levels of susceptibility than other lines; some attained their maximum early, after which susceptibility, as

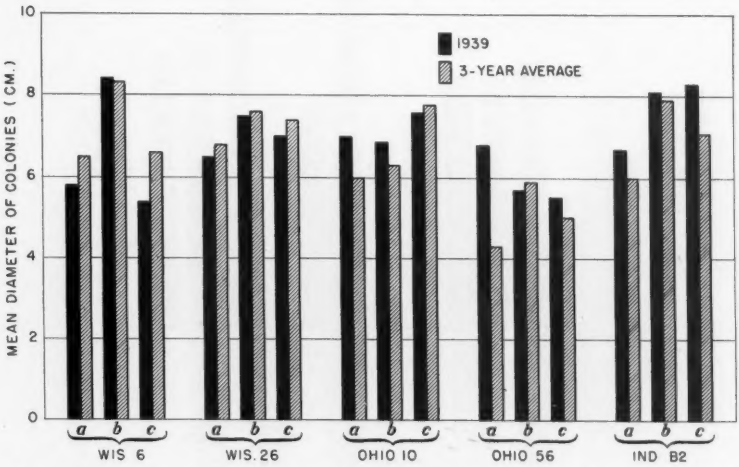

FIGURE 2.—Seasonal trends in 1939 and 3-year averages, in the growth-retarding substance of ether extracts from five inbred lines as shown by the mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts of stalks harvested in 1939 on (*a*) August 1, (*b*) August 21, and (*c*) September 8 and in 1937 to 1939, inclusive, on approximately corresponding dates.

indicated by the size of the fungus colonies, declined more or less. In other lines, maximum susceptibility was delayed until late in the season (figs. 1 and 2; table 1). Moreover, trends in short-season inbreds and long-season inbreds were likely to differ somewhat over the same three-collection period. Other complicating factors to be considered were the possible variations among plants within lines and the small number of plants in the sample collections in some years.

Differences in the seasonal change shown depended to some extent on differences in the stage of development of the plants on the date of collection. This in turn was influenced from year to year by weather conditions (*21*) (table 2) during the growing season. In some years pollination began in late July; in other years it was delayed until mid-August. When plants were collected near the time of pollination, the earlier the collection was made the greater in general was the growth-retarding effect of the ether extracts. In some lines the

seasonal trend shown varied little from year to year (fig. 2, Wis. 6 and Wis. 26). In other lines, data obtained in different years appeared at first glance to conflict. Such was the case with the extracts of Ohio 56 over a 3-year period (fig. 3), until the stage of development of the plants at the time of collection was considered. Weather conditions in 1937 and 1939 (table 2) were such that the plants were well advanced at the time of the first collections. In 1938, the temperatures were lower, with greater rainfall, and the plants developed more slowly. The first collection of that year was made 9 days before pollination

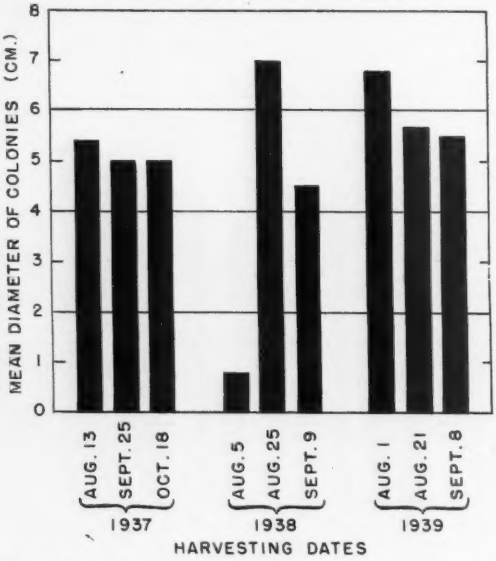

FIGURE 3.—Mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts from stalks of inbred Ohio 56, collected on different dates.

began in Ohio 56. Thus the plants of the second collection of Ohio 56 in 1938 approximated in development those of the first collections in 1937 and 1939.

TABLE 2.—*Temperature and rainfall May to August, 1934–40, at Madison, Wis.*

Year	Temperature (sum of average deviations, May to August)	Rainfall		Year	Temperature (sum of average deviations, May to August)	Rainfall	
		Total, May to August	Deviation from normal			Total, May to August	Deviation from normal
	°F.	*Inches*	*Inches*		°F.	*Inches*	*Inches*
1934	+16.8	9.22	−5.48	1938	+3.1	15.84	+1.14
1935	−3.3	14.74	+.04	1939	+11.6	8.22	−6.48
1936	+15.4	9.99	−4.71	1940	−.7	17.87	+3.17
1937	+9.0	6.80	−7.90				

The mean diameter of colonies of *Diplodia zeae*, on agars containing the ether extracts of 11 inbreds of diverse character, is shown graphically in figure 4 for each of 3 collections per season. The results

obtained during a 5-year period indicate that, on the average, maximum resistance occurred at the time of the first sample collection. Susceptibility increased to its maximum at the time of the second collection, and in this group of inbreds the mean continued at the same level through the third collection. In the case of the 5 single crosses, no differences in the general direction of their individual trends were shown during the period of sampling, although the pitch of the curves varied. In each hybrid line, as in the mean (fig. 4), maximum susceptibility was delayed until the time of the third collection.

The growth-retarding influence of the ether extracts of various inbreds was not specific for *Diplodia zeae*. The spread of colonies

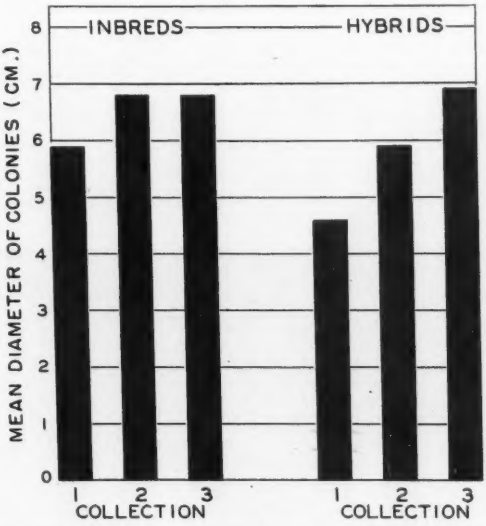

FIGURE 4.—Seasonal susceptibility of corn to stalk rot as indicated by the mean diameters of colonies of *Diplodia zeae* on agar containing ether extracts of stalks of plants collected on 3 dates each year. The means involve 11 inbred lines and 5 hybrids, differing among themselves in the number of days required to reach maturity and in degree of resistance shown in the field. Seven of the inbreds were grown for 3 successive years, between 1935 and 1939, inclusive; 1 hybrid was grown in 1935 and 4 in 1940.

of *Nigrospora sphaerica* (Sacc.) Mason (*Basisporium gallarum* Moll.) likewise was retarded more by the extract from stalks of Ill. R4 than by that from stalks of Ill. Lan (fig. 5, *A*).

The effect of the extracts on the growth of *Gibberella zeae* (Schw.) Petch (*G. saubinetii* Auct.; not *G. saubinetii* (Mont.) Sacc.) (fig. 5, *B*) also followed the general trends shown for *Diplodia zeae*. On the other hand, the spread of *G. fujikuroi* (Saw.) Wr. (*Fusarium moniliforme* Sheldon) appeared to be affected little by any of the extracts used in the agar upon which it was grown. The colonies were measurable earlier than the colonies of *Diplodia zeae*, and differences in size, occasioned by the dates of collection of the stalks or by

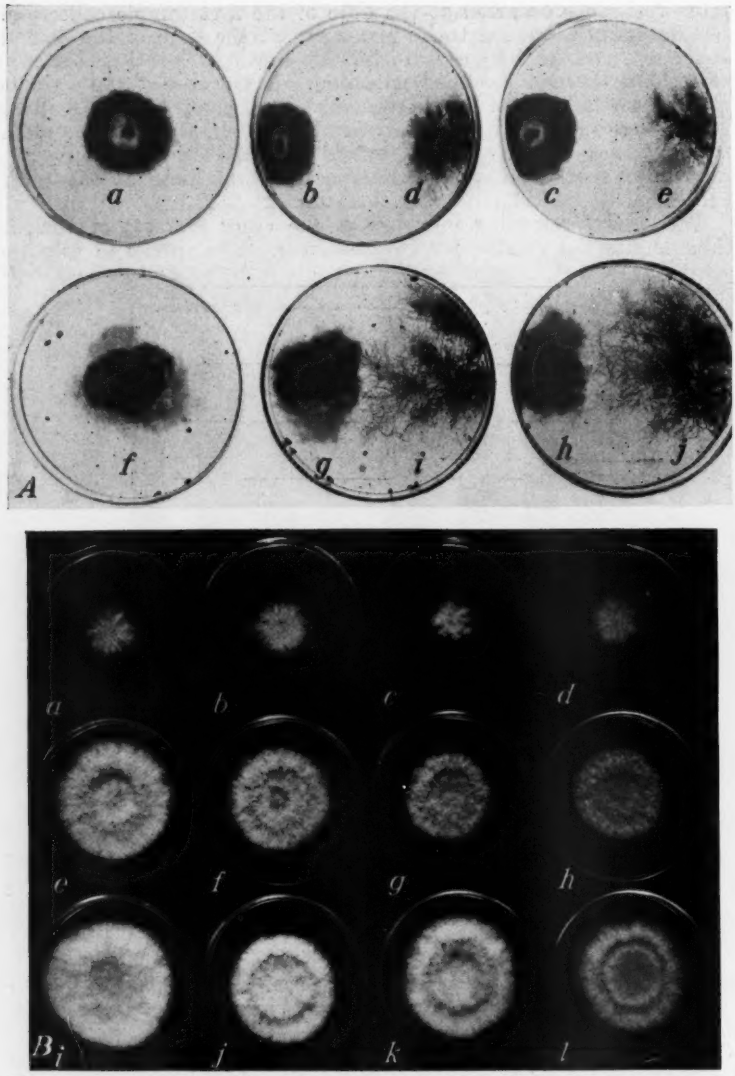

FIGURE 5.—Seven-day-old colonies of *Diplodia zeae*, *Nigrospora sphaerica*, and *Gibberella zeae* on synthetic agar containing 4 percent of ether extract of stalks of various inbreds. *A*: *a–c*, *D. zeae* on ether extract from inbred Ill. R4; *d* and *e*, *N. sphaerica*; *f–h*, *D. zeae* on ether extract from inbred Ill. Lan; *i* and *j*, *N. sphaerica*. *B*, *G. zeae* on ether extract from various inbreds: *a-d*, Ohio 56; *e*, Wis. R3; *f*, Wis. 26; *g*, Wis. 6; *h*, Wis. 23, collected August 5, 1938; *i*, Wis. R3; *j*, Wis. 26; *k*, Wis. 6; *l*, Wis. 23, collected September 21, 1938.

different strains of corn, were small. Sectoring in *G. fujikuroi* was increased by all the extracts.

Water-Soluble Fraction of Ether Extract

One test was made of the effect of a water-soluble fraction of the ether extract on the growth of *Diplodia zeae*. A 4-cc. portion of the ether extract of stalk tissues of Wis. 6 and Ohio 10, harvested on August 25, and of Ohio 56, harvested on September 9, 1937, was added to a 100-cc. portion of distilled water in each case; the mixture was well shaken, and the ether was evaporated off. The remaining filtered aqueous solutions were used instead of water in the preparation of synthetic agar (fig. 6, *e–g*). At the same time, synthetic agar prepared in the usual way received 4 percent of ether extract (fig. 6,

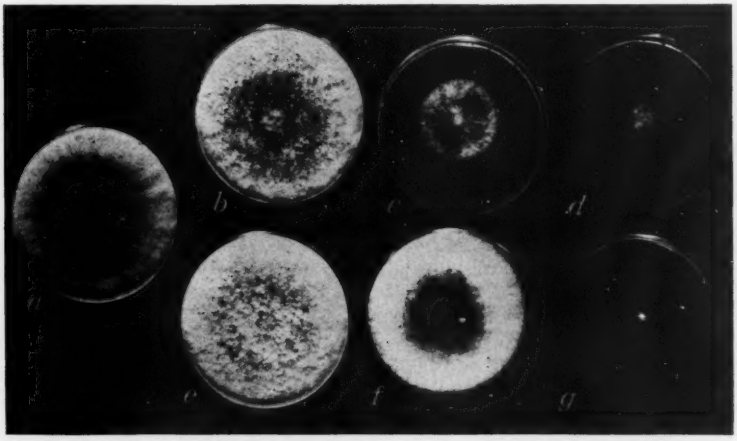

Figure 6.—Colonies of *Diplodia zeae* on synthetic agar containing ether extracts or water-soluble fractions of the extracts: *a*, Control (no extract added); *b–d*, ether extract of stalks of inbreds, Wis. 6 and Ohio 10, harvested August 25, 1937, and Ohio 56, harvested September 9, 1937, respectively; *e–g*, water-soluble fractions of the extracts from same inbreds.

b–d). Figure 6, *f* and *g*, indicates that a portion of the ether-soluble growth-retarding substance was soluble in water.

Attempted comparisons of the relative solubility in ether, alcohol, and water of the toxic substance in the cornstalk tissue were not successful, because of the large amounts of other soluble materials extracted from the stalk meal by both alcohol and water, which apparently served as nutrient material for the fungus.

Ether Extracts from Stalks of Leaf-Clipped and Defruited Plants

In 1938 and 1939, attempts were made to determine whether the ether-soluble substance in the stalk tissue could be modified either by preventing kernel formation or by partial defoliation of the growing plant. In 1938, the ear shoots were bagged and, although fertilization did not occur, the cobs and husks grew to normal size. In 1939, the

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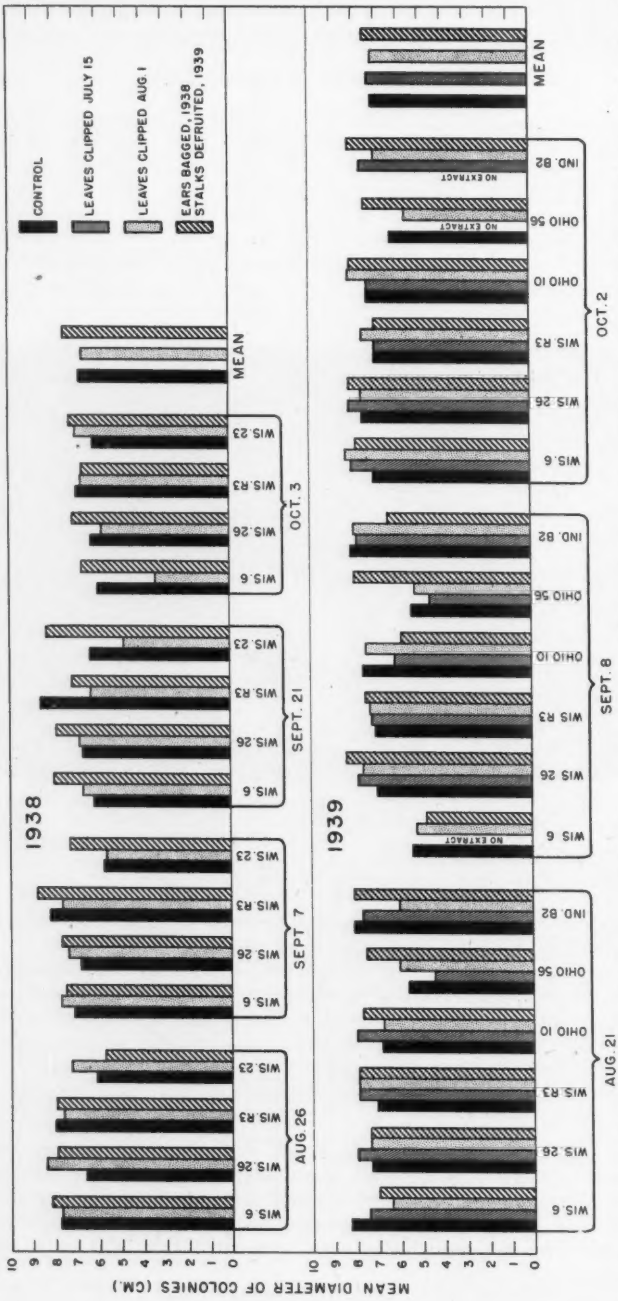

FIGURE 7.—Effect of partial defoliation and of the prevention of kernel formation on the growth-retarding substance of ether extracts of plants of various lines so treated in 1938 and 1939. Mean diameters of colonies of *Diplodia zeae* on synthetic agar containing ether extracts of inbreds Wis. 6, Wis. 26, Wis. R3, and Wis. 23, harvested on different dates in 1938; of inbreds Wis. 6, Wis. 26, Wis. R3, Ohio 10, Ohio 56, and Ind. B2, harvested on different dates in 1939; and the mean for each treatment in 1938 and in 1939.

ears were removed at pollination time. In that year the distal half of all leaves of other plants of each line were removed, some on July 15 and others on August 1. The leaves of the plants clipped on July 15 continued to elongate, so that the final result of that clipping was the removal of approximately only one-third of the normal leaf area.

Visible differences in the plants followed these treatments. On August 9, 1939, it was noted that leaf clipping appeared to have retarded normal ear development in the single-eared inbreds and to have reduced the number of ears in the two- and three-eared strains. Most of the plants of Wis. 6 and Ohio 56 whose leaves were clipped on July 15 were showing only one ear; those clipped on August 1, two ears, one of which was small; while the unclipped control plants had what promised to be two good ears in the process of development. The plants from which the ears had been removed were the most resistant to lodging; their stalks remained green longer and were harder to cut with a knife and to break than the stalks subjected to other treatments. The pith of the defruited plants of Wis. 6, and to a less degree those of Ohio 10 and Wis. R3, was mottled with brown throughout the portion of the stalk harvested. The discoloration of the cell walls and the plugging of the intercellular space were similar in appearance to the symptoms that accompany fungus invasion or wounds or that result from the overnutrition of certain cells (*11, pp. 189–190*). Platings and sections of this discolored tissue failed to reveal the presence of a fungus.

Freehand sections of defruited stalks collected in mid-September 1939, when treated with potassium iodide-iodine solution, showed the presence of considerably more starch around the bundles in the outer pith and subepidermal layers than was seen in the partly defoliated plants. In sections of stalks of Ohio 56 harvested on September 19, it was noted that starch extended farther toward the center of the stalk than in the other strains examined.

The effect of the treatments on the ether-soluble substance in the stalks of the individual lines, in 1938 and 1939, as measured by platings, is shown in figure 7. Other workers (*4, 5, 12*) have reported changes in resistance or susceptibility of plants in the field following similar treatments and have found that increased resistance accompanied changes resulting in an increase in the carbohydrate content of the stalks. In the present work there was no indication, in the erratic values obtained in both years (fig. 7), that prevention of kernel formation and the consequent increase in the carbohydrate content of the stalk consistently resulted in increased toxicity of the ether extract; nor was a uniform increase in susceptibility indicated in the stalk extracts of the partly defoliated plants as compared with those of the control plants.

Expressed Juice from Normal Stalks

Juice was expressed from stalks of Ill. R4 and Ill. Lan harvested on August 25, September 21, and October 8, 1936. The sap expressed from the plants of the first harvest was rendered sterile by passage through a Seitz filter. Spores of *Diplodia zeae* were germinated in hanging drops of the sterile juice. After 18 hours at room temperature, elongation of the germ tubes in the juice of Ill. R4 was approximately 25 percent less than that in the juice of Ill. Lan. In the stalks

harvested September 21 and October 8, when juice agar was used, a consistent difference between the two inbreds was shown by the size of the colonies. In comparable sets of plates in 1936, the diameters of all individual colonies, as well as their means, were smaller on the Ill. R4 juice agar than on the Ill. Lan juice agar (figs. 8 and 9, *A*).

Similar experiments with the fungus on juice agars of 7 inbreds were conducted in 1937. Weekly collections were made from August 13 to September 17. The method employed was that used in 1936, except that the expressed juice was centrifuged for 5 minutes instead of being filtered.

The juice from two to four plants of each inbred was used and platings were made after each collection (fig. 8). Line differences were shown in the weekly collections; and in 1937, as in 1936, colonies of

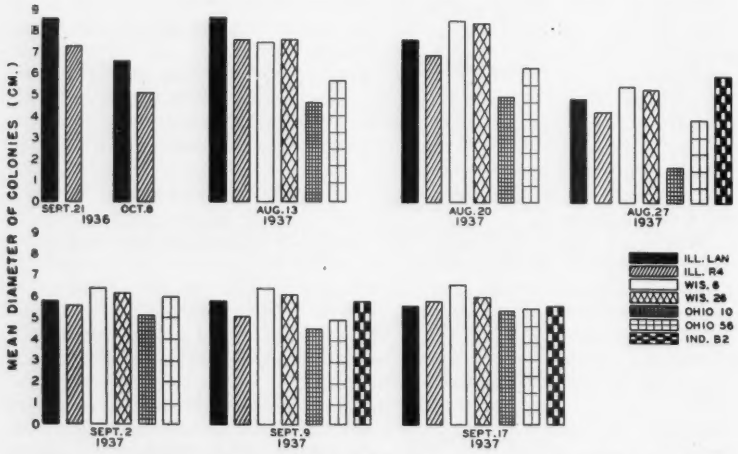

FIGURE 8.—Diameters of colonies of *Diplodia zeae* on agar containing juices of various inbreds. The sizes of the colonies may be compared for a single collection but not necessarily for different collection dates in 1937, for in that year platings were made immediately after each collection and no plating included all collections.

the fungus on the juice agars of Ill. R4 were, with one exception, smaller than those on the corresponding juice agars of Ill. Lan (fig. 8).

In order to determine if the effects of the various juices on the growth of *Diplodia zeae* could be ascribed to differences in pH, tests were made of the juices of plants collected on September 21, 1936, and on August 20, 1937. These tests gave no indication either of wide differences in the pH of the juices from the various lines or of a relation between the size of the colonies and the acidity of the medium at the time the plates were poured (table 3).

Because of an insufficient amount of juice in some samples no complete series of plates involving the juices of each line for all dates of collection was made. Hence, no comprehensive test was obtained of the seasonal change in the inhibiting action of the juices. Platings made with the juice agars of three inbreds harvested on August 13,

20, and 26 showed less retardation in the colony growth on the later dates than on August 13 (table 4). Figure 9, *B*, likewise shows a seasonal change in the early-maturing Wis. 6 and the late-maturing Ohio 10.

Similarity in the strain differences of juices and the extracts of seven inbred lines is shown in figure 10, although the plants were sampled over different periods of time. The relative susceptibility of Ill. Lan and Ill. R4 was the same for the juices as for the extracts. On the juice

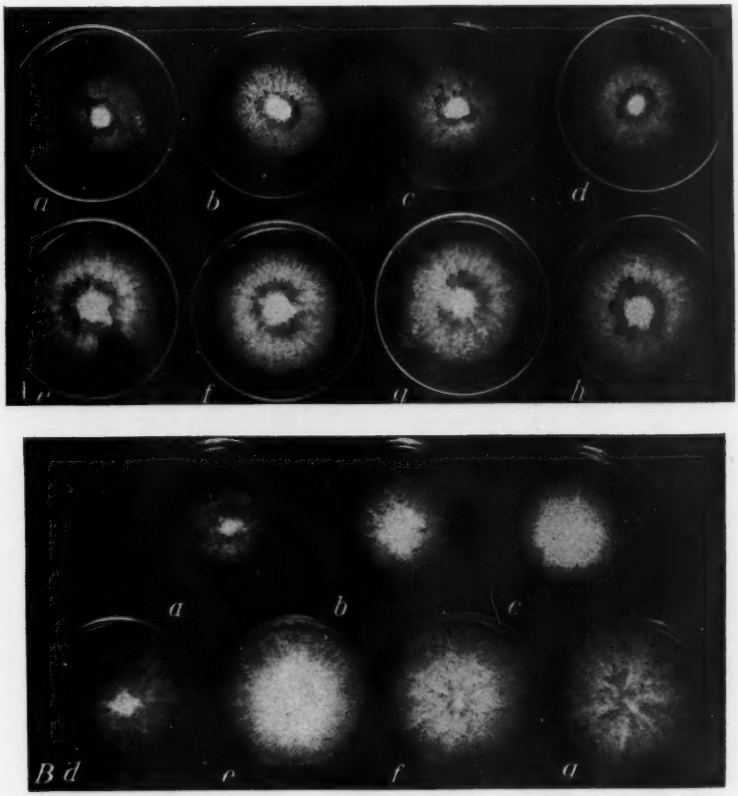

FIGURE 9.—Colonies of *Diplodia zeae* on juice agar: *A*, Colonies 3 days old on stalk juice from (*a–d*) inbred Ill. R4 and (*e–h*) inbred Ill. Lan, collected September 21, 1936; *B*, colonies 4 days old on stalk juice from (*a–c*) inbred Ohio 10, collected August 20 and September 2 and 17, 1937, respectively, and from (*d–g*) inbred Wis. 6, collected August 13 and 26 and September 2 and 17, 1937, respectively.

agars of six collections in 1937, the colonies on agar containing the juice of Ohio 10 were on the average the smallest. On the ether extracts of plants from three collections each, in 1937, 1938, and 1939, the colonies on the extracts of Ohio 56 were the smallest and those of Ohio 10 relatively large. Considering both media, the two Wisconsin inbreds gave the largest colonies and Ohio 56 the smallest.

TABLE 3.—*Diameters of colonies of Diplodia zeae grown on juice agars of plants collected September 21, 1936, and August 20, 1937, and the pH of the respective agars*

Inbred	Diameter of colonies		pH of juice agar	
	Sept. 21, 1936	Aug. 20, 1937	Sept. 21, 1936	Aug. 20, 1937
	Cm.	*Cm.*		
Ill. Lan	8.3	6.4	5.20	5.55
Ill. R4	6.7	5.6	5.27	5.46
Wis. 6		7.4		5.48
Wis. 26		7.4		5.84
Ohio 10		4.1		5.88
Ohio 56		5.4		5.69

TABLE 4.—*Trends in susceptibility in three inbred lines as indicated by the diameters of Diplodia colonies grown on juice agars of stalks harvested on different dates in 1937*

Inbred line	Diameter of colonies		
	August 13	August 20	August 26
	Cm.	*Cm.*	*Cm.*
Wis. 6	3.6		6.6
Ill. R4	4.6		4.7
Ohio 56	3.4	4.2	

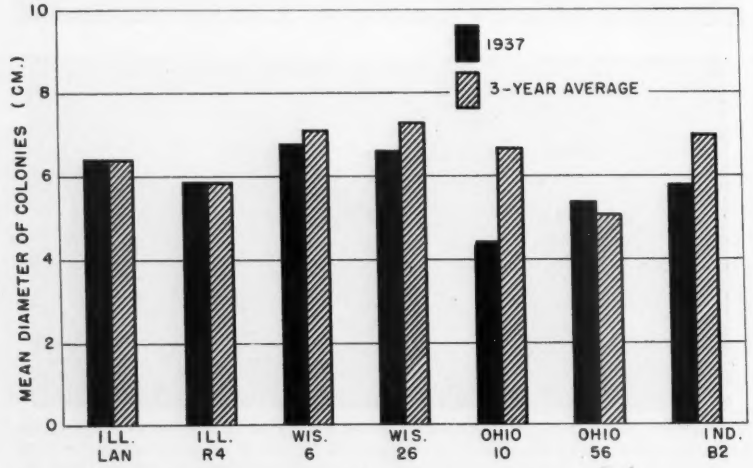

FIGURE 10.—Strain differences in resistance or susceptibility to stalk rot in various inbreds, as shown by the mean diameters of colonies of *Diplodia zeae* on juice agars of these inbreds, from plants collected at weekly intervals from August 13 to September 17, 1937, and on ether-extract agars of the same inbreds, in 3-year averages, from plants collected as follows: In 1935, August 28, September 25, and October 18; in 1936, August 20, September 15, and October 6; in 1937, August 13 and 27 and September 9; in 1938, August 5 and 25 and September 9; and in 1939, August 1 and 21 and September 8. The 3-year averages for Ill. Lan and Ill. R4 are for the years 1935 to 1937, inclusive; the others, for 1937 to 1939, inclusive.

These data appear to permit the conclusion that there is little difference in physiological susceptibility between Wis. 6 and Wis. 26. In the field, the expression of this similarity is modified by differences in the anatomical strength of the stalks and the extent of root development. Wis. 6 has a weak stalk likely to break or lodge; the stalk of Wis. 26 is strong and tends to remain erect.

SPREAD AND CHARACTER OF ARTIFICIALLY INDUCED STALK ROT

Individual lines and plants varied in their reactions to hypodermic inoculation with spore suspensions of *Diplodia zeae*, but generally resistance appeared to be relatively high until the time of pollination or shortly thereafter. Lesions resulting from inoculations made in

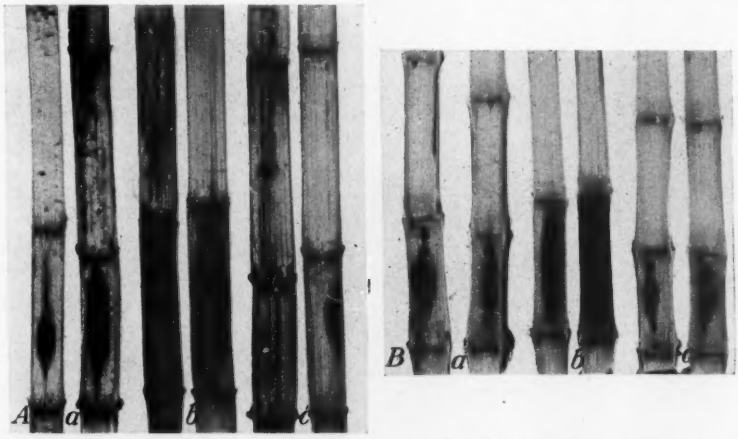

FIGURE 11.—Spread and character of lesions in two stalks of each collection of inbreds: (*A*) Wis. 6 and (*B*) Ohio 56, resulting from inoculation with *Diplodia zeae* on (*a*) August 15, (*b*) August 24, and (*c*) September 9, 1938. Stalks collected September 22, 1938.

late July or early August were smaller than those resulting from inoculations made in late August or early September, despite the advantage of the former in length of time for development before the stalks were harvested (figs. 11 and 12). Lesions resulting from the early inoculations were darker in color than those resulting from late inoculations; those resulting from the later inoculations in the more resistant strains also were darker than those in the susceptible lines inoculated on the same date. In the hybrids inoculated, maximum susceptibility generally occurred later than in the inbreds (figs. 4 and 12).

Sections through the dark lesion in the stalk of Ohio 56 (fig. 13, *A*) show a comparatively small portion of the darkened area occupied by the fungus. The discoloration of the cell walls and the plugging of the intercellular spaces extend well in advance of the hyphae (fig. 13, *B* and *C*). A lighter colored lesion in a stalk of the susceptible inbred Ill. Lan is shown in figure 14, *A*. Less retardation in the spread of the fungus and less change in the tissue of the host (fig. 14, *B* and *C*)

are indicated in this stalk. The differences shown in figures 13 and 14 were in stalks inoculated on the same date and harvested at the same time.

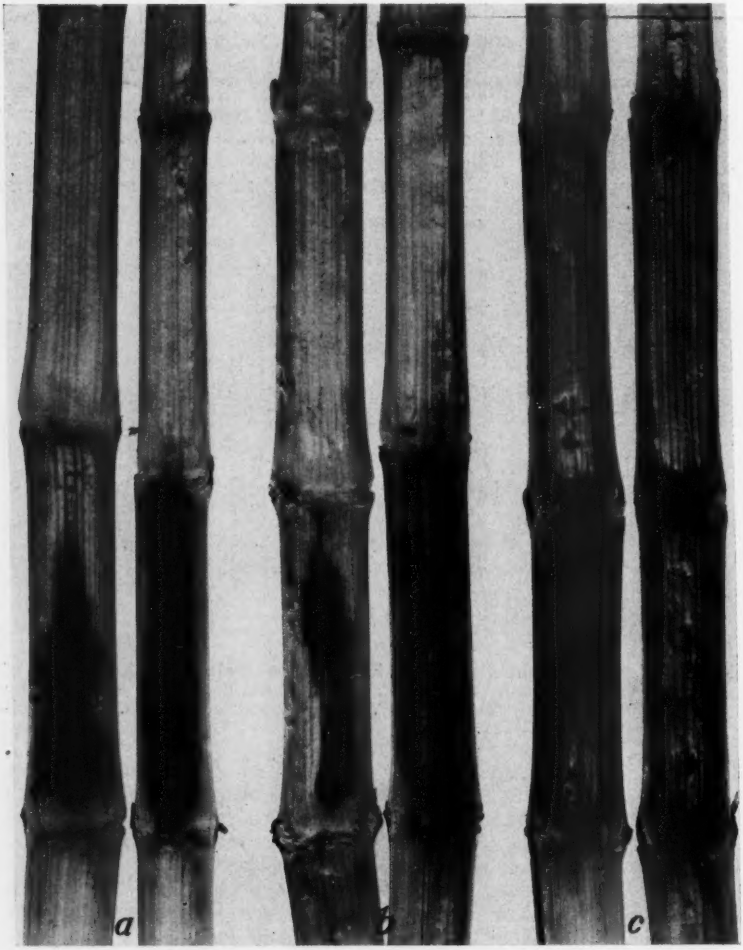

FIGURE 12.—Spread and character of lesions in stalks of hybrid Ind. WF9×Wis. 22, resulting from inoculation with *Diplodia zeae* on (*a*) July 29, (*b*) August 20, and (*c*) September 4, 1940. Stalks collected October 10, 1940.

DISCUSSION

The literature on plant diseases contains many references to disease resistance, but only a few of the later papers need be mentioned here in order to indicate some of the phases of the problems that have been investigated and the conclusions that have been drawn.

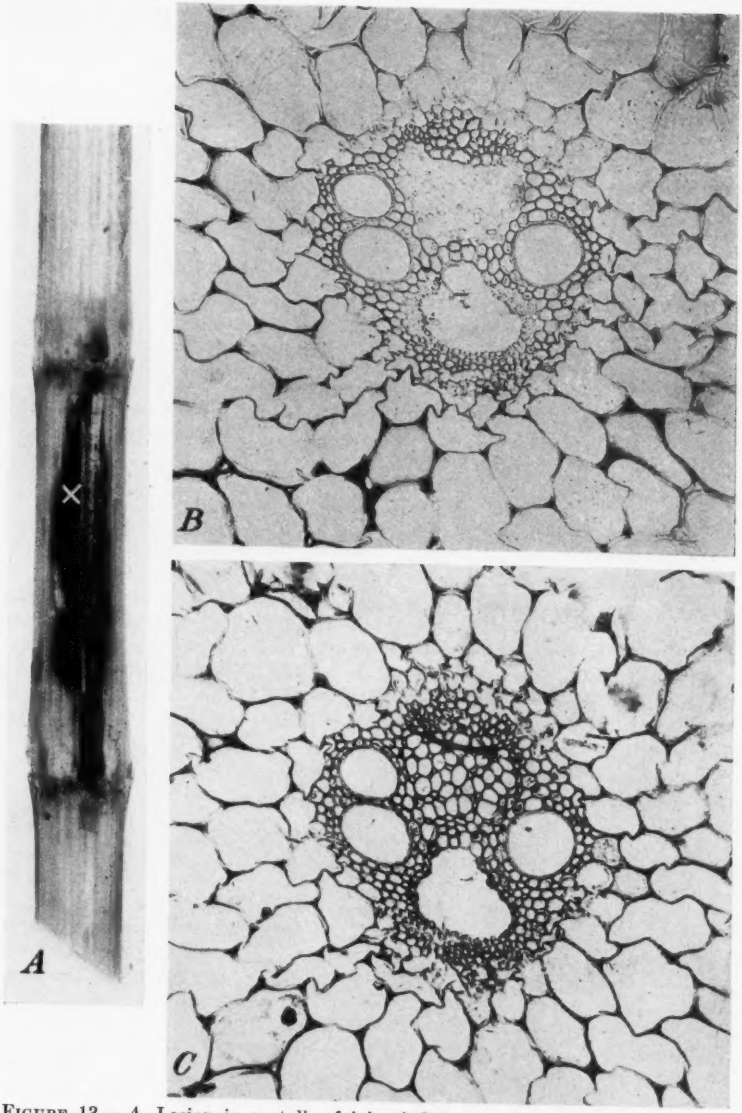

FIGURE 13.—*A*, Lesion in a stalk of inbred Ohio 56, inoculated with *Diplodia zeae* August 20 and harvested September 3, 1937. *B*, Section from location marked (x) in the dark-colored tissue in *A*, midway between puncture and node, showing plugging of intercellular spaces and discoloration of cell walls; section from fixed and embedded material, unstained, mounted in balsam. *C*, The same bundle on the next slide of the same series as section shown in *B*, stained with Flemming's triple stain to show the absence of fungus in this area. *B* and *C*, × 110.

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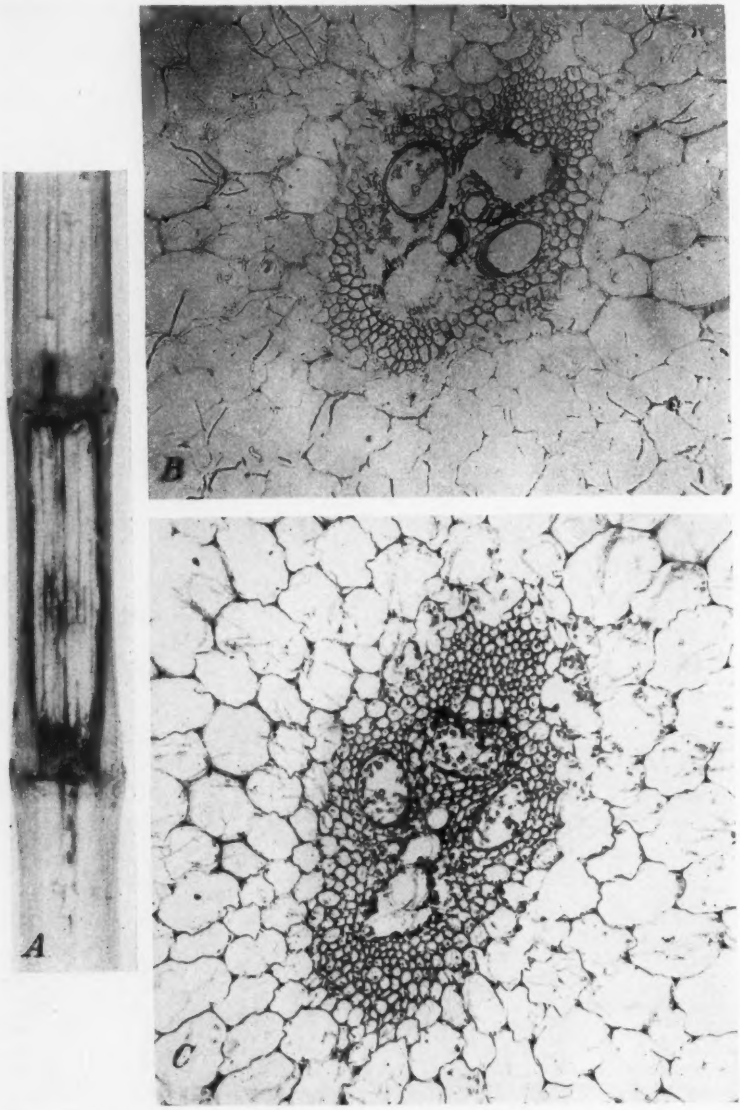

FIGURE 14.—*A*, Lesion in a stalk of inbred Ill. Lan, inoculated with *Diplodia zeae* August 20 and harvested September 3, 1937. *B*, Portion of a section from the light-colored area of the infected internode shown in *A;* little plugging of the intercellular spaces or discoloration of the cell walls has occurred; section from fixed and embedded material, unstained, mounted in balsam. *C*, Portion of another serial section of the same area as shown in *B*, stained with Flemming's triple stain to show hyphae permeating the cells of both pith and bundle. *B* and *C*, × 110.

With regard to the extent that stalk breaking indicates stalk rot, Copper et al. (*3, p. 177*) stated: "Data taken this year [1939] showed a very close relationship in some crosses and very little relationship in others."

Koehler [7] and Holbert et al. (*12*), from their observations of stalk rot in the field, agree that corn plants generally become increasingly susceptible as the ear approaches maturity.

Elliott (*7, p. 262*) found that "A change in the resistance of dent-corn plants to bacterial wilt apparently takes place in late July and August, after the plants have tasselled" and that "On dent corn, local leaf-blight lesions on maturing plants do the greatest damage."

Hart (*10*), Ivanoff and Riker (*13*), and Ranker (*18*) concluded that more than one type of resistance may have been present in the plants they studied. Smith and Walker (*20*) ascribed resistance to yellows to antagonistic chemical substances or physiolgical qualities in the roots of the cabbage plants. Other workers have linked resistance to the presence in host tissue of phenolic compounds (*6, 17*), protocatechuic acid (*1*); tannin (*2*), berberine (*9*), and catechol (*15*), substances known to be toxic to fungi. Moore (*16*) found water-soluble, growth-inhibiting substances in roots resistant to phymatotrichum root rot. Ezekiel and Fudge (*8, p. 786*), working with the same disease, stated: "The general immunity of monocots to root rot is due at least in part to the presence in the roots . . . of minute quantities of acidic, ether-soluble substances, possibly organic acids or esters." Reynolds (*19*), after experimenting with water extracts of different parts of flax plants, suggested that the quantitative results obtained may be correlated with the actively functioning protoplasm in the different parts.

Conclusions reached in the present study have been in substantial agreement with those in several of the articles cited. The juices and ether extracts of stalks harvested before or shortly after pollination were found to retard the growth of *Diplodia zeae* in culture to a greater degree generally than those of the later harvests. This accords with the results of the writers' inoculations in the field and with the occurrence of stalk rot as observed by Koehler and Holbert (*14*) and Holbert et al. (*12*).

Results were not altogether uniform from year to year, nor was there uniformity in the weather conditions under which the plants developed. In comparing results, therefore, one must remember that plants were not necessarily at similar stages of development on the same calendar dates in different years, nor were all lines at the same stage of maturity at any one time of collection. Despite these limiting circumstances, certain lines held their position of relative resistance or susceptibility with regard to the one factor of the resistance complex under consideration. During the years 1937 to 1939, in comparisons of several inbreds, the extracts of Ohio 56 showed on the average the smallest colonies of *Diplodia zeae* in each year. It may be noted here also that, under field conditions in three locations in Illinois in 1939, this inbred was fifth in a group of 48 inbreds, ranked according to their resistance to damage from diplodia stalk rot as determined in single crosses. In the test plot in southern

[7] KOEHLER, B. CORN DISEASE INVESTIGATIONS IN ILLINOIS. *In* Reports of the Fourth and Fifth Corn Improvement Conferences of the North Central Region, pp. 7-8. Washington, D. C., 1942. [Processed.]

Illinois, it held first place; in northern Illinois near De Kalb, it was tenth in the list.[8]

Immunity from stalk rot was not found in any of the lines used for study; nor does it appear that the degree of resistance observed in strains in the field should be ascribed wholly to substances within the tissue that tend to retard fungus growth, or to the anatomical strength of the stalks. Both factors contribute to the well-being of the plant and are combined in different proportions in various lines. The seasonal change in resistance seems to be largely of a physiological nature. Differences between lines in field performance are influenced by both physiological and morphological factors. Advantageous as a good root system and a strong stalk are to the corn plant, they do not of themselves appear to insure resistance to or immunity from stalk rot, or to be combined necessarily with high physiological resistance. Ind. B2 has a stiff stalk and a good root system, but it has been described as susceptible to ear and stalk rots. On the other hand, Ohio 56 [9] is listed as resistant to stalk rot, but "stalk may break in late September."

After pollination there was a period in the life of each plant when the growth-retarding substance in the stalk reached its lowest level. This took place soon after pollination in some lines; in others it was delayed until late in the season. Consequently the pitch, as well as the height, of the curve of increasing susceptibility differed in individual lines during the period of sampling, and in some instances it had changed in direction before the third collection. It is not known whether this increase in the toxicity of the extract late in the season was due to the presence of the substance found in the stalks before pollination, for the chemical nature of these materials has not been determined. It seems probable, however, that changes in direction were not so much an indication of radical differences in the trend of the curve of susceptibility as of differences in timing and in the speed with which various lines passed through one or more phases of their development.

SUMMARY

Data are given for 11 inbred lines and 5 single crosses of corn (*Zea mays*) investigated from 1935 to 1940 for the presence in the stalks of an ether- or sap-soluble substance that retards the growth of *Diplodia zeae* in culture and for seasonal changes in the growth-retarding substance. The ether extracts of inbreds and hybrids collected before or shortly after pollination generally retarded growth of the fungus to a greater degree than those of later collections. Certain lines retained more of the growth-retarding substance throughout the maturation of the plant than did others, indicating a physiological type of resistance not necessarily associated with strength of stalk or morphological resistance.

Extracts that were effective in retarding the spread of *Diplodia zeae* also retarded the growth of *Gibberella zeae* and *Nigrospora sphaerica* but had little influence on the spread of *G. fujikuroi*.

The growth-retarding substance in the extract was stable to heat and soluble to some extent in water.

[8] KOEHLER, B., BOLIN, O., and COPPER, R. R. CORN INBREDS RANKED ACCORDING TO THEIR RESISTANCE TO DAMAGE FROM DIPLODIA STALK ROT AS DETERMINED IN SINGLE CROSSES IN 1939. Ill. Agr. Expt. Sta. Dept. Agron., 3 pp. 1940. [Processed.]

[9] [JENKINS, M. T., reporter.] REPORT OF THE FIRST CORN IMPROVEMENT CONFERENCE, HELD AT CHICAGO, ILLINOIS, DECEMBER 3, 1937. 40 pp. Washington, D. C. 1938. [Processed.] (See pp. 13, 24, 31.)

Removal of the ears did not consistently increase, nor did clipping the leaves decrease, the growth-retarding effect of the ether extracts of the plants so treated.

Expressed juice of the stalks contained a growth-retarding substance that showed seasonal change and differed in the lines tested much as did the ether extracts.

Results obtained by inoculating various inbreds and hybrids with *Diplodia zeae* also indicated that resistance was relatively high in all lines until the time of pollination and for shorter or longer periods in different lines thereafter. The spread of the fungus was restricted in the dark lesions of resistant plants and was impeded but little in the lighter colored lesions of susceptible tissue.

The chemical nature of the growth-retarding substance is unknown, but the suggestion is made that differences shown in the physiological resistance of the various lines may be due not so much to radical differences in the general trend of the curve of susceptibility as to differences in timing and in the speed with which lines pass through the various phases of their development.

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DIFFERENCES IN THE COMPOSITION OF THE FRUITS OF CUCURBITA VARIETIES AT DIFFERENT AGES IN RELATION TO CULINARY USE [1]

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INTRODUCTION

According to Safford (*10*) [2] pumpkin and squash were used as food by the prehistoric inhabitants of the Americas. At present they are widely grown for canning, for the city market, for home consumption, and for stock feed. The cultural methods employed in their production as crop plants have been described by Jones and Rosa (*9*) and by Thompson (*14*). The fruits are often stored for winter use, and considerable study has been given to methods of storage and to the chemical changes that occur in the fruits during the storage period (*6*, *8*). The importance of the crop for canning has stimulated considerable work on the composition of the fruits of different varieties (*6*, *7*, *11*, *13*, *16*) and on the changes in composition that take place during growth and development (*8*). Their composition as food products has been summarized by Chatfield and Adams (*4*) and Winton and Winton (*15*).

A study of the published reports upon the chemical composition of pumpkins makes it apparent that, in addition to variations attributable to climatic and soil conditions, there are consistent inherent differences in composition among varieties and also significant differences between the various stages of maturity in any given variety. For the most part, previous investigations have been concerned with small groups of varieties, and the studies have not been continued over the entire life period of the fruits.

The purpose of the present study was to determine how the composition of a large number of *Cucurbita* varieties differed at different ages from the time of bloom to the end of their storage life. Numerous cooking and canning tests were made in order to ascertain how the changes in composition affected their culinary use. To reduce the error arising from variations in seasonal conditions the work was continued over a period of 4 years.

MATERIALS AND METHODS

CULTURAL METHODS AND VARIETIES

The varieties for these studies were grown on a sandy loam of good fertility at the Arlington Experiment Farm, Arlington, Va. They were grown in rows 6.6 feet apart with plants approximately feet apart in the row. The seeds of all varieties except three were

[1] Received for publication August 18, 1943.
[2] Italic numbers in parentheses refer to Literature Cited, p. 136.

obtained from American seedsmen; Charles Naudin, Iron Bark, and Faan Kwa were of foreign origin. The planting in each of the 4 years was made on June 10. This permitted the early vegetative development of the plants to occur during the warmer part of the summer. The young plants were periodically dusted with insecticides. Shallow cultivation to destroy weeds was continued as long as the plants were small enough not to be injured by it. The vegetative growth of most varieties completely covered the ground by late July or early August.

Two varieties were grown the first year, an additional 12 the second year, 13 more the third, and 9 more the fourth, making in all 36 varieties. Of these, 8 belonged to the species *Cucurbita pepo* L., 6 to *C. moschata* Poir, and 22 to *C. maxima* Duchesne (table 1).

Descriptions of all but a few of the varieties have been given by Castetter and Erwin (*3*). The fruits of the varieties of each group varied in shape, smoothness, and color of the surface, in color and texture of the flesh, and in many other characteristics. No varieties of the summer squash type were used, but both pumpkins and squashes of the so-called winter squash types were included.

TABLE 1.—*Varieties used of each species and number of years each was grown*

Species and variety	Years grown	Species and variety	Years grown
Cucurbita pepo:	*Number*	*C. maxima*—Continued.	*Number*
Connecticut Field	3	Golden Hubbard	4
Fordhook	2	Improved Hubbard (Green Hubbard)	3
Fort Berthold	2	Iron Bark	1
Golden Oblong	3	King of the Mammoths	3
New England Pie	3	Kitchenette (Baby Hubbard)	1
Omaha	3	Mammoth Chili	3
Table Queen	3	Mammoth Whale	2
Winter Luxury	3	Marblehead	1
C. maxima:		Sibley	1
Arikara	3	Warren	2
Banana	2	Winnebago	2
Blue Hubbard	2	*C. moschata:*	
Boston Marrow	2	Faan Kwa	1
Charles Naudin	1	Green Striped Cushaw	2
Chicago Warted Hubbard	1	Japanese Pie	2
Delicious	1	Large Cheese	4
Essex Hybrid	2	Mammoth Golden Cushaw	3
French Turban (Acorn)	1	Tennessee Sweet Potato	2
Gilmore	2		
Golden Delicious	3		

ANALYTICAL PROCEDURE

To obtain comparable material for the analyses, blossoms were tagged at the time of opening. The plants generally began flowering and setting fruit in August; the field was gone over at intervals of 1 or 2 days, and tags showing the date of blooming were attached to the young fruits. Samples were usually collected on the date of blooming and 10, 20, 30, 40, and 60 days later, thus making a series of 6 samples for each variety in each year in which it was grown. The total number of series of samples was 79. The 60-day samples in nearly all cases were taken in October. The crop was then harvested and placed in common storage at 40° to 50° F.

The sampling was continued on the stored material at 90, 120, and 165 days from date of bloom. The last samples were taken the latter part of February, when many of the fruits appeared to be nearing the end of their storage life. In 6 of the 79 series, sampling was discontinued for one reason or another before the end of the storage period.

At date of flowering 15 to 30 entire fruits were used in the samples; at all other sampling periods only the wall, or edible portion of the fruit, was included. For each of these samples fractional parts of 3 to 6 fruits were used. The material was chopped into small pieces, which were thoroughly mixed. Duplicate 100-gm. samples were then weighed and placed in glass jars; sufficient 95-percent alcohol was added to make the final concentration 75 to 80 percent. The alcohol was then heated to boiling, the boiling was continued for 2 or 3 minutes, after which the lids were clamped down upon the jars, sealing them and excluding most of the air until the analytical work was begun.

The samples were prepared for analysis by extracting the material with alcohol in order to separate the sugars, acids, tannins, and other soluble material from the acid-hydrolyzable polysaccharides, pectin, and fiber. The residue was dried, weighed, and used for the determination of the acid-hydrolyzable polysaccharides. A separate sample of the fresh material was dried for the determination of total nitrogen.

The alcohol-soluble solids were determined by taking triplicate 25-ml. aliquots of the alcoholic extract, evaporating the alcohol at 60° C. on a water bath, completing the drying in a vacuum oven over calcium oxide at 75° under a negative pressure of 28 inches of mercury, and weighing.

The methods used in the determination of the various constituents were those given by the Association of Official Agricultural Chemists (*2*). The volumetric permanganate method was used for sugars, the Loewenthal-Procter method for astringent materials, titration with 0.1 N sodium hydroxide for acids, the ferrous chloride method for nitrates, and the Gunning-Arnold method as modified to include nitrates for the total nitrogen.

EXPERIMENTAL RESULTS

EFFECT OF DIFFERENT FACTORS ON THE COMPOSITION OF CUCURBITA FRUITS

An examination of the detailed analytical results showed rather large differences in the extent to which many of the constituents of the fruits varied. To determine the significance of these variations analyses of variance were made for the 36 varieties grown 1 year, for the 27 grown 2 years, and for the 14 grown 3 years; these results are not entirely independent because the 1-year results included the varieties grown for 2 and 3 years and the 2-year results included the varieties grown for 3 years.

The data used in the analysis of variance are those obtained in the samplings up to and including those at 60 days from date of blooming, at which stage the fruits of all varieties were mature. The series of samples obtained during storage were incomplete in a few instances, and the omission of the storage data makes the results strictly comparable for all varieties. The grouping of the varieties according to the number of years in which they were grown greatly simplified the computations.

In addition to the mean squares, the coefficients of variability were calculated for use in comparing the variability of the constituents. The results for the fresh weight of the fruit and for each of the chemical constituents for the three groups of data are given in table 2.

TABLE 2.—*Results of statistical analysis of the data from 36 Cucurbita varieties grown for 1, 2, or 3 years and harvested at different stages of maturity*

Item and source of variation	3-year results [1]			2-year results [1]			1-year results [1]		
	Degrees of freedom	Mean square	Coefficient of variability	Degrees of freedom	Mean square	Coefficient of variability	Degrees of freedom	Mean square	Coefficient of variability
Total solids:									
Variety	13	155.756**	29.62	26	81.837**	25.47	35	36.831**	24.85
Year	2	17.017**	3.84	1	66.196**	4.49			
Age	5	176.045**	19.53	5	257.090**	19.80	5	190.957**	21.39
Variety × year	26	3.489	6.27	26	2.258	4.23			
Variety × age	65	12.329**	18.63	130	6.448**	15.99			
Year × age	10	3.611	3.96	5	2.651	2.61			
Error	130	2.867	12.71	130	2.263	9.47	175	4.838	20.14
Soluble solids:									
Variety	13	7.040**	12.40	26	4.699**	12.33	35	1.926**	11.54
Year	2	3.959**	3.65	1	8.365**	3.23			
Age	5	28.826**	15.56	5	44.750**	16.69	5	28.154**	16.68
Variety × year	26	.631**	5.25	26	.420**	3.69			
Variety × age	65	1.351**	12.15	130	.806**	11.42			
Year × age	10	.719**	3.47	5	.465*	1.70			
Error	130	.230	7.09	130	.196	5.63	175	.428	12.17
Total sugar:									
Variety	13	4.532**	15.02	26	3.071**	14.91	35	1.109**	13.41
Year	2	4.963**	6.16	1	8.828**	4.96			
Age	5	37.642**	26.84	5	61.382**	29.23	5	34.212**	28.16
Variety × year	26	.511**	7.13	26	.383*	5.45			
Variety × age	65	.949**	15.36	130	.581**	14.50			
Year × age	10	.878**	5.80	5	1.057**	3.97			
Error	130	.221	10.48	130	.222	8.97	175	.313	15.93
Reducing sugar:									
Variety	13	1.354**	10.61	26	.768**	9.59	35	.483	10.93
Year	2	.387	2.22	1	1.690**	2.79			
Age	5	11.956**	19.56	5	19.536**	21.20	5	14.989**	23.01
Variety × year	26	.191	5.63	26	.212	5.03			
Variety × age	65	.535**	14.92	130	1.202**	26.81			
Year × age	10	.219	3.74	5	.326	2.74			
Error	130	.155	11.36	130	.147	9.38	175	.277	18.51
Acidity:									
Variety	13	.01229**	15.54	26	.08468**	16.19	35	.00474**	16.20
Year	2	.00681**	4.54	1	.03080**	6.06			
Age	5	.08690**	25.63	5	.12388**	27.16	5	.07797**	24.84
Variety × year	26	.00092	6.01	26	.00101	5.59			
Variety × age	65	.00169**	12.88	130	.00133*	14.34			
Year × age	10	.00206*	5.58	5	.00584**	5.90			
Error	130	.00100	14.05	130	.00094	12.05	175	.00139	19.59

Total astringency:									
Variety	13	.000185**	12.36	26	.000134**	10.94	35	.000052**	10.77
Year	2	.000057	2.67	1	.000111**	1.95			
Age	5	.002796**	29.80	5	.004416**	27.51	5	.001504**	21.85
Variety × year	26	.000050*	8.00	26	.000046	6.41			
Variety × age	65	.000052**	12.89	130	.000037	12.86			
Year × age	10	.000140**	8.30	5	.000468**	8.96			
Error	130	.000031	14.06	130	.000032	12.00	175	.000028	17.61
Nitrate nitrogen:									
Variety	13	.000219**	19.43	26	.000117**	25.70	35	.000100**	23.08
Year	2	.002572**	26.12	1	.008967**	44.21			
Age	5	.000174**	10.75	5	.000155**	13.00	5	.000613**	21.64
Variety × year	26	.000036*	11.17	26	.000027	12.35			
Variety × age	65	.000035*	17.39	130	.000034	30.82			
Year × age	10	.000049*	8.02	5	.003070*	8.73			
Error	130	.000022	19.35	130	.000050	28.96	175	.000025	26.00
Total nitrogen:									
Variety	13	.025019**	16.92	26	.015531**	16.44	35	.009494**	16.83
Year	2	.045959**	9.29	1	.100147**	8.18			
Age	5	.398371**	43.24	5	.478714**	40.01	5	.260941**	33.34
Variety × year	26	.001910*	6.83	26	.001581	5.24			
Variety × age	65	.002150**	11.45	130	.000916	8.93			
Year × age	10	.007240**	8.24	5	.013857	6.81			
Error	130	.001273	12.47	130	.002220	13.90	175	.002055	17.50
Acid-hydrolyzable polysaccharides:									
Variety	13	73.400**	72.34	26	37.116**	58.90	35	17.881**	59.94
Year	2	3.213	5.94	1	25.480**	9.57			
Age	5	66.718**	42.77	5	85.962**	39.31	5	65.396**	43.32
Variety × year	26	2.196	17.70	26	1.140	10.32			
Variety × age	65	7.158**	50.51	130	3.560**	40.79			
Year × age	10	.931	7.14	5	6.554**	10.85			
Error	130	1.453	32.18	130	.988	21.49	175	2.779	32.84
Weight of fruit:									
Variety	13	66.936**	60.27	26	35.626**	55.13	35	19.444**	54.89
Year	2	12.024**	10.02	1	10.882**	5.98			
Age	5	172.429**	60.00	5	230.059**	61.44	5	178.242**	62.81
Variety × year	26	1.853**	14.18	26	.891	8.72			
Variety × age	65	6.169**	40.92	130	3.717**	39.82			
Year × age	10	2.219**	9.62	5	2.599**	6.53			
Error	130	.797	20.70	130	.725	17.59	175	2.459	43.64

[1]*Significant; **highly significant.

The data show highly significant variations attributable to differences in varieties and age. The mean squares that are significant in the 3-year group of varieties are generally also significant in the 2-year and 1-year groups. The variation due to differences in years is in general relatively small and in a few cases not significant. For most constituents the age of the fruit is the source of greater variation than variety. There is considerable unexplained variance, obviously due to sampling error, resulting from the large differences in the individual fruits. The acid-hydrolyzable polysaccharides are generally the most variable of the chemical constituents determined, the variation being even greater in many cases than that of the total fresh weight of the fruit. Other constituents whose variability appears to be large and important are total solids and total sugars. The variability of the content of acids, astringent materials, soluble solids, and reducing sugars is in most cases smaller, for both principal factors and their interactions, than that of the other constituents.

RELATION OF WEIGHT AND CHEMICAL CONSTITUENTS TO AGE

In order to obtain an understanding of the general relation between the age and size of the fruit and its composition, the results at each stage of maturity and each storage period for all varieties and all years were averaged. The curves up to and including 60 days from date of bloom (fig. 1) are means for a series of 79 samples. Six of these were discontinued at various times during the storage period, so that the points on the curves at 90, 120, and 165 days are means for 77, 75, and 73 samples, respectively. It is obvious that the various curves could not have been measurably different had the entire series been carried to the end of the storage period.

The increase in the fresh weight of the fruit is represented by a curve (fig. 1) similar to the general growth curve. The maximum fresh weight of the fruit was reached at about 60 days of age, after which there was a slow decrease in storage. Because of the rather high humidity and moderately low temperature of the storage room this decrease in weight caused by drying was small. The respiration was evidently fairly rapid, since total solids decreased more rapidly than the fresh weight. The decrease in total solids of the fresh material began before the fruit was harvested, was greatly accelerated in the first month of storage (60 to 90 days of age), and then slowed down. About two-thirds of the total loss in storage occurred in the first third of the storage period.

As would be expected, the course of the chemical changes of the several constituents differed greatly. The changes in no constituent exactly paralleled those in any other, but in several constituents they were somewhat similar. The direction of the curves for acidity, total astringency, and total nitrogen is very similar. The curves for soluble solids and total sugar are nearly parallel except during the first 10 days' growth, when the changes in the sugar were pronounced and the soluble solids changed little. During the same period the total solids decreased, while the acid-hydrolyzable polysaccharides increased; for the remainder of the life of the fruit the direction of the changes in the two curves was very similar. The curves indicate that the acid-hydrolyzable polysaccharides reached a maximum somewhat earlier than the total solids and the fresh weight. The direction of the curve for total nitrogen is almost the reverse of that for nitrate

nitrogen; that is, when the total nitrogen increased, the nitrate nitrogen decreased.

The changes in most of the constituents during the growing period, especially up to the 40-day stage, were much more pronounced than those that took place during storage. The most pronounced change in any constituent during storage was in the acid-hydrolyzable poly-

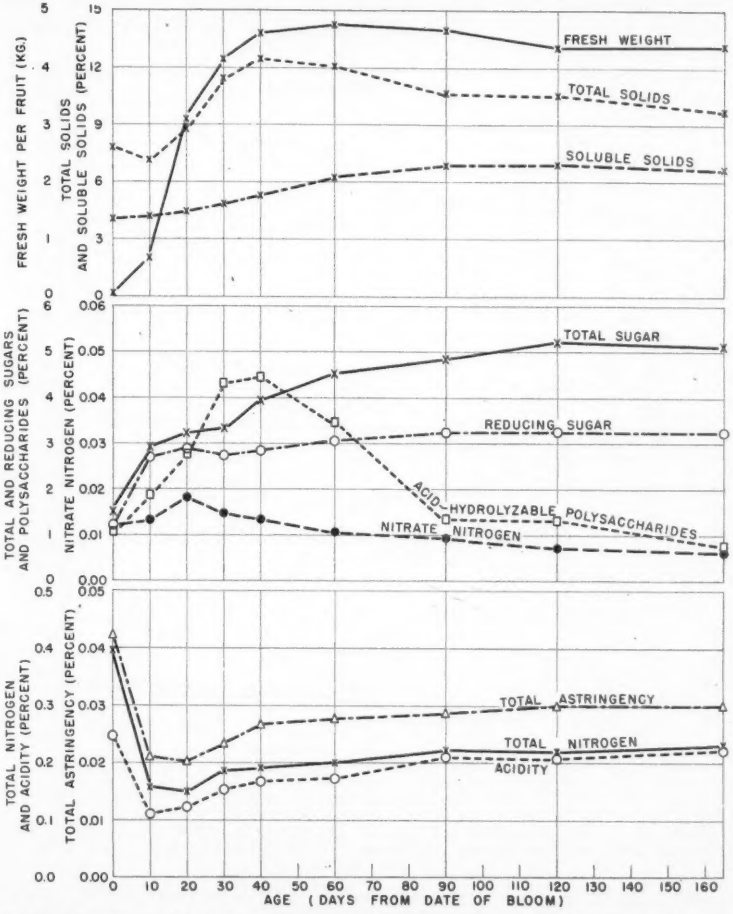

FIGURE 1.—General relation between the fresh weight, or size, of *Cucurbita* fruits, their content (fresh-weight basis) of various chemical constituents, and their age.

saccharides. Next in importance was the change in total sugar. Total sugar increased while acid-hydrolyzable polysaccharides decreased. Total nitrogen and astringent substances increased somewhat during storage as a result of greater loss of other constituents, whereas nitrate nitrogen decreased.

The changes in the composition are of such importance in relation to the problems connected with the use of the fruit as food and in

studies of the physiological processes that it seemed advisable to compare the results expressed on the fresh-weight basis with those expressed on the dry-weight basis. The results have therefore been calculated as percentages of the dry matter present.

The curves on the dry-weight basis for the soluble solids, total sugar, and reducing sugars are somewhat similar; that is, they increase from flowering to the 10-day stage and then decrease to the 30- or 40-day stage, after which they increase to the end of the storage

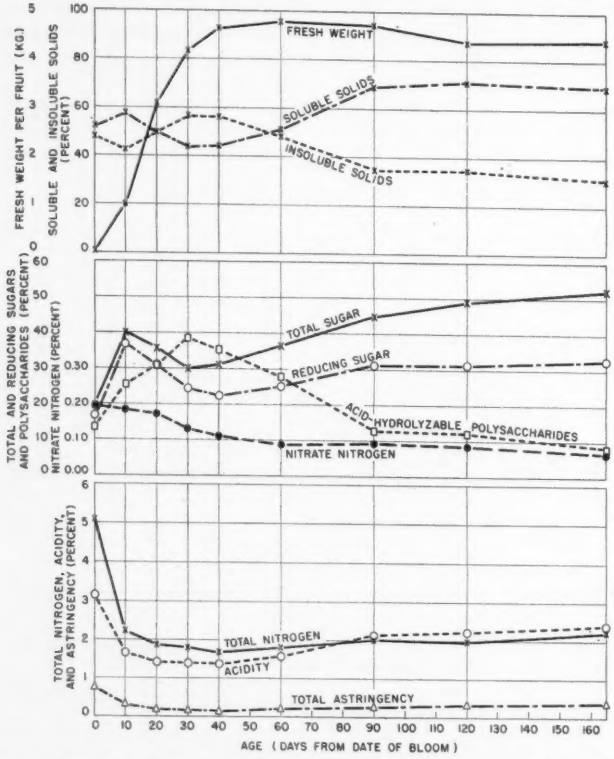

FIGURE 2.—General relation between the fresh weight, or size, of *Cucurbita* fruits, their content (dry-weight basis) of various chemical constituents, and their age.

period (fig. 2). The decrease between the 10- and the 30-day stages is due very largely to the increase in the acid-hydrolyzable polysaccharides during this period. These curves are rather strikingly different from those on the fresh-weight basis, as is evident from a comparison of figures 1 and 2. The sugars and soluble solids are lowest at the 30- and 40-day stages, when the acid-hydrolyzable polysaccharides are highest. It is evident that the starch-sugar ratio is highest at this period.

The curves for total and nitrate nitrogen and acidity are somewhat similar to the corresponding curves on the fresh-weight basis. In most cases the changes that occur before maximum fresh weight has

been attained are much more rapid and more pronounced than those that take place subsequently to or during storage.

While the percentage composition is of great importance because of its relation to the edible quality and food value of the product, the yield, or the total amount of the constituents produced, determines to an even greater extent the value of the crop and must be given con-

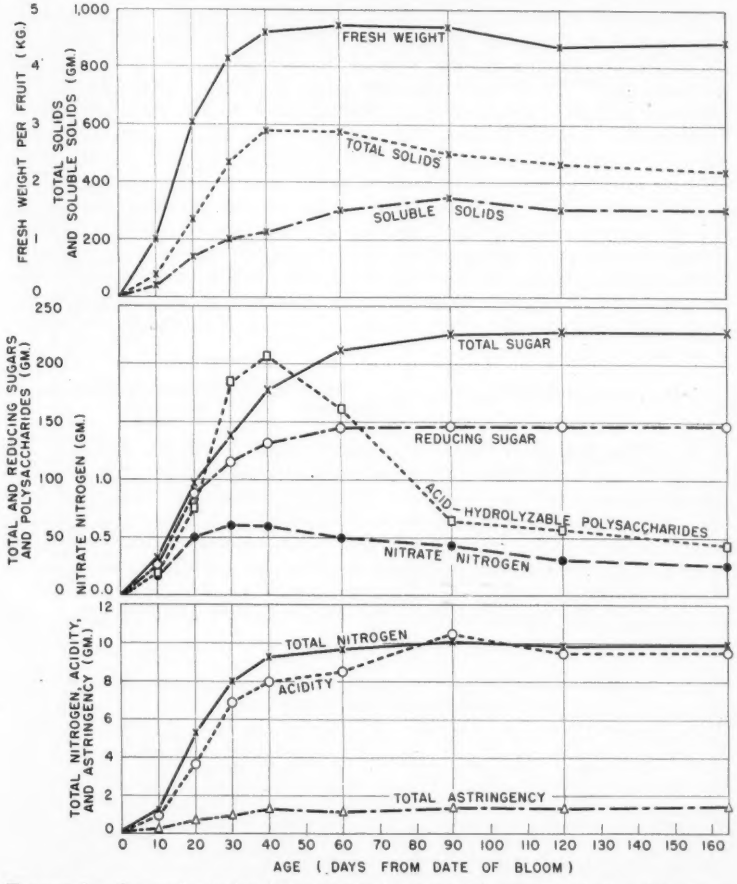

FIGURE 3.—General relation between the fresh weight, or size, of *Cucurbita* fruits, the total amounts per fruit of various chemical constituents, and their age.

sideration. Consequently, it seemed desirable to study the relation of the age of the material to the total amounts of the constituents produced. The data obtained afford an idea of the comparative rates of change in the total amounts of the constituents per fruit. The fresh-weight per fruit was multiplied by the percentage of each of the constituents determined in the analyses and the results were plotted (fig. 3).

The curves in figure 3 show the total amount of each constituent per fruit if one assumes that the entire fruit consists of wall tissue. Since the wall tissue makes up such a large part of the fruit, these curves probably correspond rather closely to those for actual values. All the curves except those for total solids and acid-hydrolyzable polysaccharides are somewhat similar, rising slowly from the flowering to the 10-day stage, very rapidly to the 30- or 40-day stage, and then progressively more slowly to the 40- or 60-day stage. The curve for total solids is almost a straight line from the 10-day to the 40-day stage; that is, for the greater part of the period of growth of the fruit. Consequently, it is evident that dry matter is added to the fruit at an almost constant rate over the greater part of the growing period. During the storage period, that is, from the 60-day stage onward, there are only moderate or inconsequential changes in the total amount per fruit of soluble solids, total sugars, total nitrogen, and acidity. All of these, except soluble solids, which decrease toward the end of the storage period, tend to increase slightly. The greatest change in the total amount per fruit during storage was in the acid-hydrolyzable polysaccharide content. The change in the total-solids content was also quite marked. There was a small but important decrease in fresh weight and a definite though less pronounced decrease in nitrate nitrogen.

RANKING OF VARIETIES ACCORDING TO CONTENT OF THEIR CHEMICAL CONSTITUENTS

In the discussion of the analysis of variance it was pointed out that there are large and highly significant variations attributable to differences among varieties. To determine the general rank of the different varieties, the variety means were obtained by averaging the results at all stages of maturity for all years. These average values are much more reliable for general comparison than those obtained only at the time of harvest. However, the ranking is essentially the same in both cases. The comparisons of the varieties for the different average constituents are shown in table 3.

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*

Constituent, numerical rank, and variety	Content	Constituent, numerical rank, and variety	Content
Total solids:	*Percent*	Total solids—Continued.	*Percent*
1. Table Queen	15.7	21. French Turban	9.1
2. Delicious	15.2	22. Japanese Pie	9.0
3. Banana	13.4	23. Golden Oblong	8.8
4. Improved Hubbard	13.1	24. New England Pie	8.7
5. Sibley	12.9	25. Marblehead	8.2
6. Golden Hubbard	12.9	26. Green Striped Cushaw	8.1
7. Fordhook	12.8	27. Fort Berthold	7.9
8. Blue Hubbard	12.3	28. Mammoth Golden Cushaw	7.8
9. Winnebago	12.1	29. Large Cheese	7.6
10. Chicago Warted Hubbard	11.8	30. Mammoth Chili	7.5
11. Gilmore	11.7	31. Omaha	7.4
12. Essex Hybrid	11.5	32. Winter Luxury	7.3
13. Kitchenette	11.4	33. Mammoth Whale	7.3
14. Golden Delicious	10.8	34. Faan Kwa	7.0
15. Arikara	10.5	35. Connecticut Field	6.7
16. Iron Bark	10.4	36. King of the Mammoths	6.4
17. Warren	10.1	Soluble solids:	
18. Tennessee Sweet Potato	9.4	1. Table Queen	8.1
19. Boston Marrow	9.2	2. Fordhook	7.2
20. Charles Naudin	9.1	3. Sibley	6.6

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*—Continued

Constituent, numerical rank, and variety	Content
Soluble solids—Continued.	*Percent*
4. Delicious	6.4
5. Chicago Warted Hubbard	6.3
6. Improved Hubbard	6.25
7. Winnebago	6.2
8. Blue Hubbard	6.2
9. Gilmore	6.1
10. Iron Bark	6.0
11. Golden Hubbard	5.9
12. Kitchenette	5.8
13. Golden Delicious	5.8
14. Essex Hybrid	5.7
15. Golden Oblong	5.7
16. Warren	5.6
17. New England Pie	5.6
18. Charles Naudin	5.5
19. Tennessee Sweet Potato	5.5
20. Arikara	5.4
21. Japanese Pie	5.4
22. Boston Marrow	5.3
23. Omaha	5.2
24. French Turban	5.0
25. Fort Berthold	5.0
26. Mammoth Golden Cushaw	4.9
27. Marblehead	4.9
28. Mammoth Whale	4.9
29. Large Cheese	4.8
30. Green Striped Cushaw	4.8
31. Winter Luxury	4.7
32. Faan Kwa	4.6
33. Mammoth Chili	4.6
34. King of the Mammoths	4.6
35. Mammoth Whale	4.4
36. Connecticut Field	4.4
Total sugars:	
1. Table Queen	6.0
2. Fordhook	5.3
3. Sibley	4.9
4. Iron Bark	4.6
5. Winnebago	4.4
6. Chicago Warted Hubbard	4.3
7. Delicious	4.3
8. Blue Hubbard	4.3
9. Banana	4.2
10. Gilmore	4.2
11. Improved Hubbard	4.1
12. Japanese Pie	4.0
13. Golden Oblong	4.0
14. Tennessee Sweet Potato	4.0
15. Kitchenette	4.0
16. Charles Naudin	3.9
17. Golden Hubbard	3.9
18. Warren	3.8
19. Essex Hybrid	3.8
20. New England Pie	3.7
21. Golden Delicious	3.6
22. Arikara	3.5
23. Fort Berthold	3.5
24. Omaha	3.5
25. Boston Marrow	3.4
26. Green Striped Cushaw	3.4
27. French Turban	3.4
28. Mammoth Golden Cushaw	3.3
29. Faan Kwa	3.3
30. Large Cheese	3.3
31. Marblehead	3.3
32. Mammoth Chili	3.2
33. King of the Mammoths	3.2
34. Winter Luxury	3.0
35. Connecticut Field	2.9
36. Mammoth Whale	2.9
Acid-hydrolyzable polysaccharides:	
1. Delicious	6.5
2. Table Queen	4.7
3. Banana	4.5
4. Golden Hubbard	4.2
5. Improved Hubbard	4.0
6. Sibley	3.7
7. Golden Delicious	3.7

Constituent, numerical rank, and variety	Content
Acid-hydrolyzable polysaccharides—Con.	*Percent*
8. Blue Hubbard	3.5
9. Essex Hybrid	3.5
10. Kitchenette	3.4
11. Winnebago	3.2
12. Gilmore	3.0
13. Fordhook	3.0
14. Arikara	2.9
15. Chicago Warted Hubbard	2.7
16. Iron Bark	2.6
17. Warren	2.5
18. French Turban	2.4
19. Boston Marrow	2.0
20. Tennessee Sweet Potato	1.9
21. Japanese Pie	1.8
22. Charles Naudin	1.6
23. Green Striped Cushaw	1.8
24. Marblehead	1.8
25. Mammoth Whale	1.6
26. Mammoth Chili	1.6
27. Mammoth Golden Cushaw	1.5
28. Large Cheese	1.4
29. New England Pie	1.3
30. Faan Kwa	1.1
31. Golden Oblong	1.1
32. Omaha	1.0
33. Fort Berthold	1.0
34. Winter Luxury	1.0
35. King of the Mammoths	.9
36. Connecticut Field	.6
Total nitrogen:	
1. Delicious	.298
2. Fordhook	.277
3. Table Queen	.271
4. Kitchenette	.270
5. Golden Hubbard	.259
6. Banana	.258
7. Gilmore	.251
8. Blue Hubbard	.250
9. Golden Delicious	.249
10. Improved Hubbard	.248
11. Arikara	.237
12. Chicago Warted Hubbard	.235
13. Winnebago	.235
14. Essex Hybrid	.230
15. Sibley	.226
16. Iron Bark	.214
17. Warren	.212
18. Boston Marrow	.211
19. Charles Naudin	.211
20. Japanese Pie	.205
21. Marblehead	.204
22. Tennessee Sweet Potato	.203
23. Omaha	.195
24. New England Pie	.192
25. French Turban	.190
26. Golden Oblong	.183
27. Mammoth Golden Cushaw	.182
28. Winter Luxury	.177
29. Green Striped Cushaw	.175
30. King of the Mammoths	.170
31. Mammoth Whale	.169
32. Faan Kwa	.167
33. Fort Berthold	.164
34. Large Cheese	.163
35. Mammoth Chili	.162
36. Connecticut Field	.148
Nitrate nitrogen:	
1. Marblehead	.0236
2. Chicago Warted Hubbard	.0224
3. Kitchenette	.0184
4. Boston Marrow	.0169
5. Sibley	.0160
6. Arikara	.0157
7. Mammoth Chili	.0144
8. King of the Mammoths	.0143
9. Golden Delicious	.0140
10. Charles Naudin	.0137
11. Mammoth Golden Cushaw	.0133

TABLE 3.—*Rank of 36 Cucurbita varieties according to the average content of various constituents (fresh-weight basis)*—Continued

Constituent, numerical rank, and variety	Content	Constituent, numerical rank, and variety	Content
Nitrate nitrogen—Continued.	*Percent*	Acids—Continued.	*Percent*
12. Faan Kwa	0.0132	25. Japanese Pie	0.172
13. Gilmore	.0127	26. Mammoth Golden Cushaw	.172
14. Blue Hubbard	.0126	27. Green Striped Cushaw	.168
15. Essex Hybrid	.0123	28. Warren	.163
16. Mammoth Whale	.0115	29. Fort Berthold	.162
17. French Turban	.0113	30. Marblehead	.161
18. Winnebago	.0111	31. Connecticut Field	.160
19. Golden Hubbard	.0110	32. French Turban	.156
20. Fort Berthold	.0107	33. Boston Marrow	.155
21. Delicious	.0105	34. Mammoth Chili	.127
22. Iron Bark	.0105	35. King of the Mammoths	.126
23. Connecticut Field	.0104	36. Mammoth Whale	.121
24. Improved Hubbard	.0101	Total astringency:	
25. Banana	.0100	1. Banana	.034
26. Warren	.0100	2. Fordhook	.034
27. Green Striped Cushaw	.0092	3. Gilmore	.033
28. New England Pie	.0084	4. Improved Hubbard	.033
29. Omaha	.0084	5. Delicious	.032
30. Large Cheese	.0076	6. Golden Hubbard	.032
31. Winter Luxury	.0073	7. Table Queen	.032
32. Japanese Pie	.0072	8. Blue Hubbard	.031
33. Tennessee Sweet Potato	.0066	9. Arikara	.030
34. Golden Oblong	.0065	10. Kitchenette	.030
35. Table Queen	.0050	11. Essex Hybrid	.030
36. Fordhook	.0042	12. Large Cheese	.030
Acids:		13. Sibley	.030
1. Table Queen	.261	14. Chicago Warted Hubbard	.030
2. Fordhook	.253	15. Golden Delicious	.029
3. Delicious	.213	16. Winnebago	.029
4. Banana	.210	17. French Turban	.028
5. Improved Hubbard	.209	18. Warren	.028
6. Chicago Warted Hubbard	.209	19. Iron Bark	.028
7. Iron Bark	.205	20. New England Pie	.028
8. Golden Hubbard	.204	21. Omaha	.028
9. Kitchenette	.200	22. Golden Oblong	.027
10. Winnebago	.200	23. Japanese Pie	.027
11. Omaha	.195	24. Marblehead	.027
12. Charles Naudin	.195	25. Boston Marrow	.026
13. Arikara	.192	26. Charles Naudin	.026
14. Blue Hubbard	.191	27. Faan Kwa	.026
15. Sibley	.187	28. Winter Luxury	.026
16. Essex Hybrid	.185	29. Tennessee Sweet Potato	.025
17. Gilmore	.185	30. Mammoth Whale	.024
18. New England Pie	.184	31. Connecticut Field	.023
19. Winter Luxury	.181	32. Fort Berthold	.023
20. Tennessee Sweet Potato	.180	33. Green Striped Cushaw	.023
21. Golden Oblong	.177	34. Mammoth Chili	.023
22. Faan Kwa	.177	35. Mammoth Golden Cushaw	.023
23. Golden Delicious	.176	36. King of the Mammoths	.022
24. Large Cheese	.176		

TOTAL SOLIDS

Table 3 shows a wide range in the total-solids content of the different varieties, the extremes being 6.4 and 15.7 percent of the fresh weight. About half of the group fall within the range of 8 to 12 percent solids.

Among those that rank high in total solids are a number of widely grown varieties, such as Table Queen, Delicious, Banana, Improved Hubbard, Sibley, and Golden Hubbard; among those low in total solids are King of the Mammoths, Connecticut Field, Mammoth Whale, Winter Luxury, Omaha, and Mammoth Chili, all widely grown. The species *Cucurbita pepo* contains some varieties that are among the highest in solids and others that are among the lowest, Table Queen and Fordhook being high and Connecticut Field and Winter Luxury being low. Among the varieties of the species *C. moschata* none were outstandingly high in total solids, but Faan

Kwa and Large Cheese were among the lowest. In the *C. maxima* group Improved Hubbard and Banana were high, while King of the Mammoths and Mammoth Chili were low.

A fairly good negative correlation was found between size of fruits at maturity and total-solids content; in other words, the large fruits were generally higher in moisture than the small fruits.

Soluble Solids

The ranking of the varieties for soluble solids is somewhat like that for total solids (table 3). Table Queen, Fordhook, Delicious, Improved Hubbard, and Sibley are among the highest in both total and soluble solids, whereas Connecticut Field, King of the Mammoths, Faan Kwa, Mammoth Chili, and Mammoth Whale are among the lowest.

Total Sugars and Acid-Hydrolyzable Polysaccharides

The ranking of the varieties in respect to total sugar content is similar to that for acid-hydrolyzable polysaccharide content (table 3). Varieties that on an average are high in sugar are generally high in acid-hydrolyzable polysaccharides. High acid-hydrolyzable polysaccharide content in fruit at or approaching maturity is correlated with high sugar content late in the storage period. The polysaccharide content is particularly high in Delicious and Table Queen and very low in Connecticut Field, while the sugar content is particularly high in Table Queen and low in Mammoth Whale and Connecticut Field.

Total Nitrogen

The varieties with a high total-solids content (table 3) were also generally somewhat higher in total nitrogen than the varieties with a low total-solids content. On the other hand, if the results are calculated on the dry-weight basis the varieties that are high in total solids are found to be low in total nitrogen. In other words, there is a smaller proportion of nitrogenous materials in the total solids of those varieties high in dry matter than in those low in dry matter, the principal constituents that account for the high total solids being sugars and acid-hydrolyzable polysaccharides.

Nitrate Nitrogen

Among the varieties low in nitrate nitrogen (table 3) are Fordhook, Table Queen, Golden Oblong, and Tennessee Sweet Potato, while among those highest in nitrates are Marblehead, Chicago Warted Hubbard, Kitchenette, and Boston Marrow. These differences are important because of the corroding effect of nitrates on tin containers (*5*).

Titratable Acidity

Although the differences in acid content were often statistically significant, they seemed to be unimportant from a practical standpoint. Among the varieties that were highest in acidity are Table Queen, Fordhook, Delicious, Banana, Improved Hubbard, Chicago Warted Hubbard, and Golden Hubbard (table 3); Mammoth Whale, King of the Mammoths, and Mammoth Chili were exceptionally low, their acid content being on an average less than half of that of the highest.

Total Astringency

The astringency of the fruits of all the varieties was very low, and differences, although sometimes statistically significant, appear to be entirely unimportant from the standpoint of the quality of the products. Among those highest (table 3) were Banana, Fordhook, Gil-

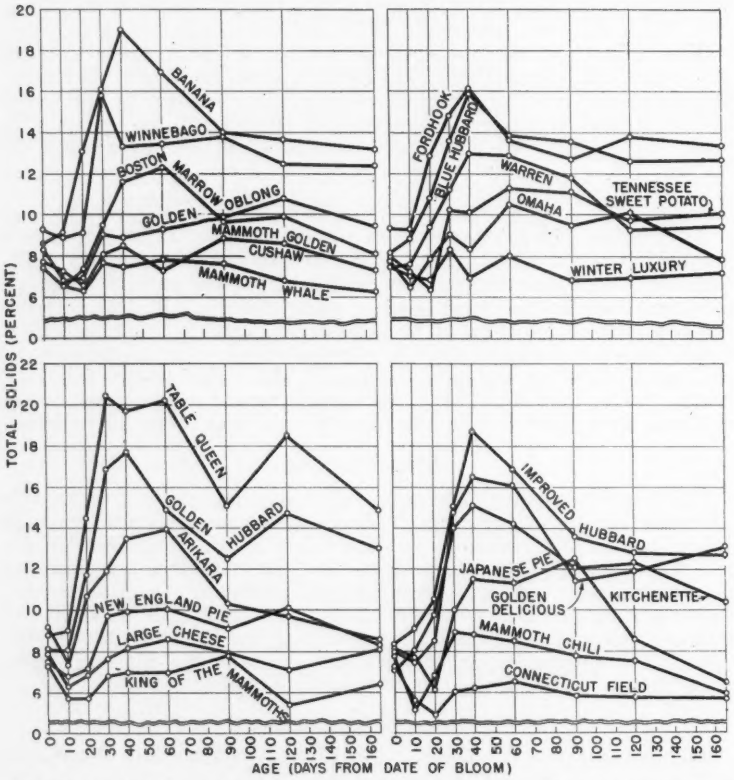

Figure 4.—Relation between the average total-solids content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in the different varieties.

more, and Improved Hubbard, the last being more widely grown than the others.

Varietal Differences at Different Ages

Although the means shown in table 3 indicate the general ranking of the varieties as to composition, it is often more important to know how they rank at a particular stage of maturity or storage. From table 2 it is apparent that there is a rather large, highly significant variance attributable to the interaction of variety and age. This variance (variety $\times$ age) shows that the varieties do not differ to the same extent at all stages of maturity.

Total Solids

The changes in the percentage of total solids in the fresh material with increase in age are comparatively small in certain varieties and rather large in others (fig. 4). The percentages of total solids in all varieties are nearly the same at the time of flowering. In Mammoth Whale, Winter Luxury, King of the Mammoths, Mammoth Golden Cushaw, and Connecticut Field they change relatively little during the entire period of growth and throughout storage. On the other hand,

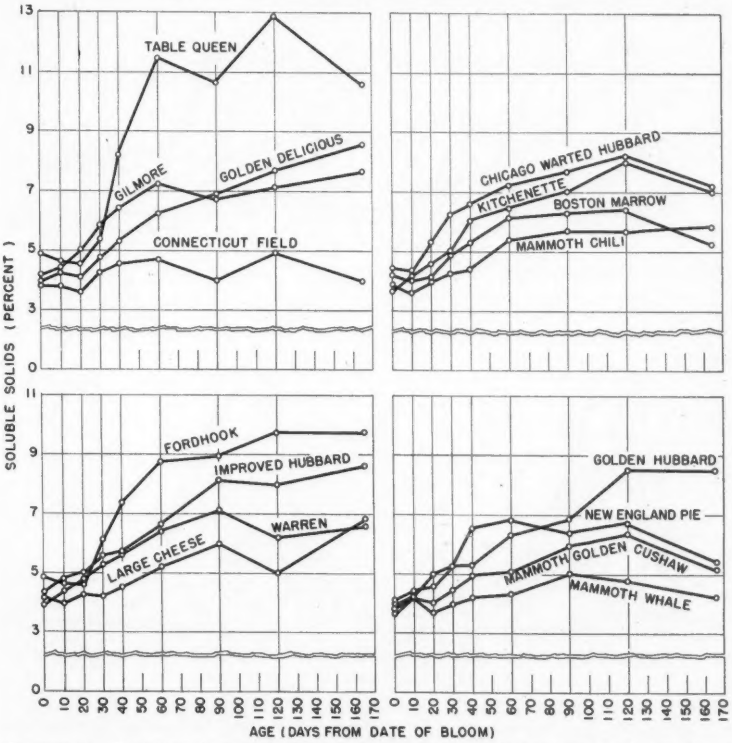

Figure 5.—Relation between the average soluble-solids content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

the total solids (percent) in Table Queen, Fordhook, Banana, Improved Hubbard, Blue Hubbard, and Golden Delicious increase tremendously from the flowering stage to the 30- or 40-day stage, but they decline slowly during storage. Between these two groups there are a number of varieties that are intermediate in the extent of the changes during development.

Soluble Solids

The differences among the varieties in respect to changes in soluble solids are less striking than in total solids, but the differences are often important (fig. 5), as in the case of Connecticut Field and Table

Queen. There were comparatively small changes in Mammoth Whale and Mammoth Chili with increase in age, but rather large changes occurred in Fordhook, Chicago Warted Hubbard, Table Queen, and Golden Hubbard. New England Pie, Boston Marrow, and Mammoth Whale, showed a tendency to decrease in soluble

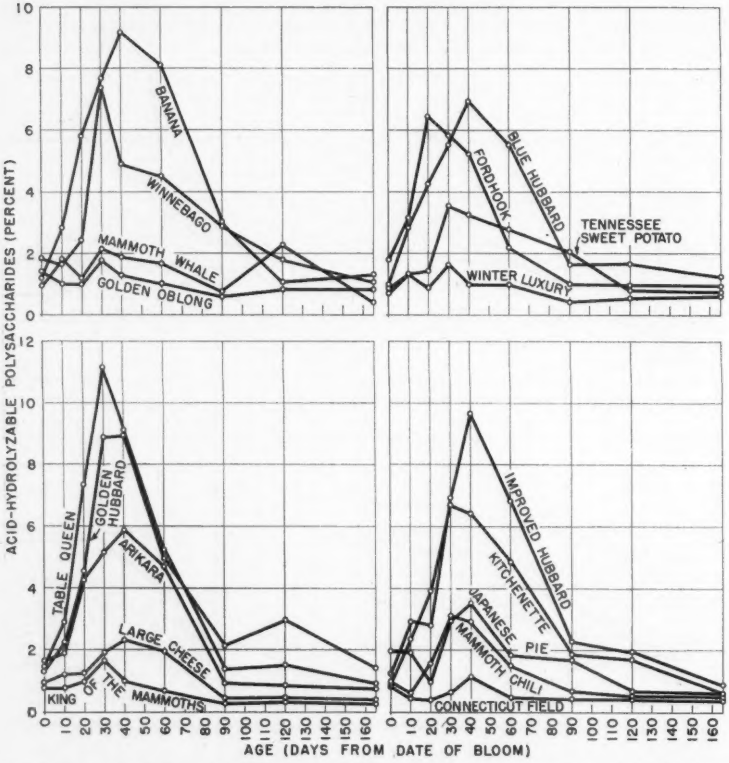

FIGURE 6.—Relation between the average acid-hydrolyzable polysaccharides content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

solids during storage, whereas Golden Delicious, Golden Hubbard, Improved Hubbard, and Fordhook showed a tendency to increase.

ACID-HYDROLYZABLE POLYSACCHARIDES

The variance attributable to the interaction of age with variety is greater in the acid-hydrolyzable polysaccharides than in any other constituent of the total solids. This is accounted for by the differences in the changes of the varieties with increase in age, which is often very striking (fig. 6). Examples of this extreme contrast are Banana and Golden Oblong, Table Queen and King of the Mammoths, Blue Hubbard and Winter Luxury, Improved Hubbard and Connecticut

Field. Between the varieties that are highest and those that are lowest are others of intermediate type, among which may be mentioned Tennessee Sweet Potato, Arikara, Japanese Pie, and Mammoth Chili.

Total Sugars

Since sugar content at the time of flowering is very low, the differences in varieties at this stage are comparatively small (fig. 7).

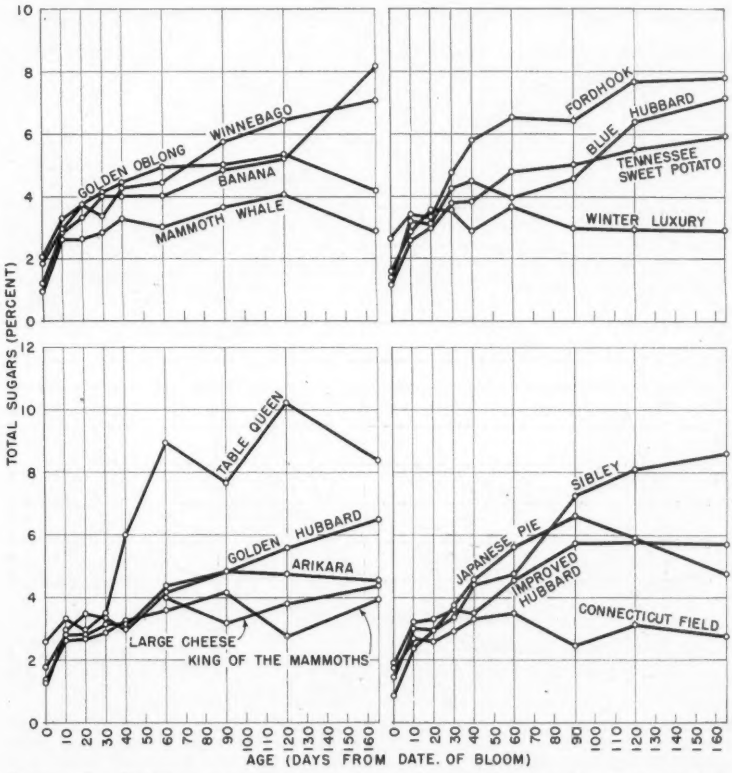

FIGURE 7.—Relation between the average sugar content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

With increasing age, however, the differences between varieties may become much more pronounced. Certain varieties, such as Connecticut Field, Winter Luxury, Mammoth Whale, and King of the Mammoths, never become very high in sugar, whereas other varieties, such as Table Queen, Fordhook, Banana, and Blue Hubbard, are much higher than the average in the older stored material (90 to 120 days of age). There is generally a similar increase in sugar in all varieties up to the 30- or 40-day stage, after which the low-sugar varieties cease to increase while the others continue to increase far

into the storage period. The reason for this is obvious: those varieties that are low in acid-hydrolyzable polysaccharides do not increase greatly in sugar during storage, while those high in acid-hydrolyzable polysaccharides eventually become high in sugar as a result of the conversion of starch into sugar.

Total and Nitrate Nitrogen, Acidity, and Astringency

The variance attributable to the interaction of age and variety in the case of the other consitituents is generally not as significant or as

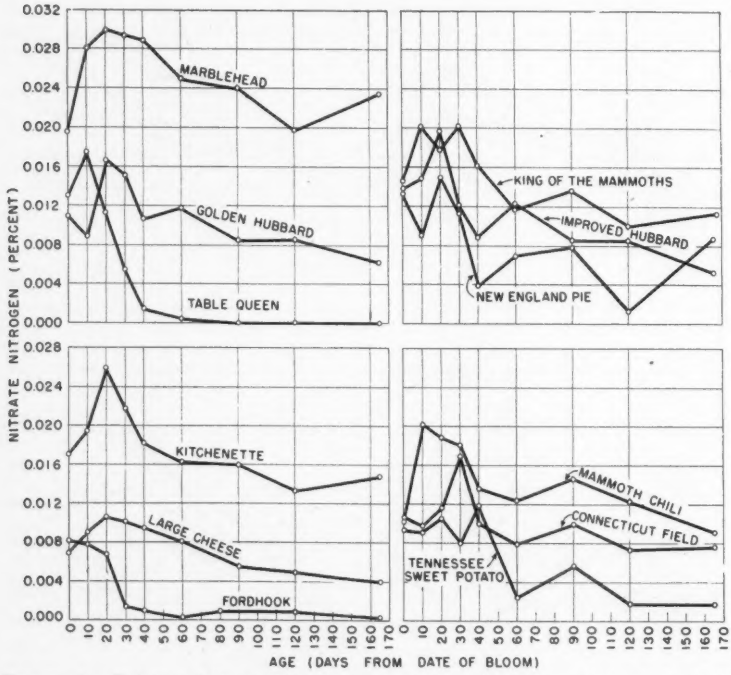

FIGURE 8.—Relation between the average nitrate nitrogen content of the fruit of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

important as with the acid-hydrolyzable polysaccharides, sugars, and total solids. It does appear to be important in the case of nitrate nitrogen (fig. 8) in a few varieties. Table Queen and Fordhook, for example, have appreciable amounts of nitrate nitrogen in their tissues only in the early developmental stages. From about the 40-day stage onward nitrates are negligible in these varieties, whereas in Marblehead, Golden Hubbard, and Kitchenette appreciable quantities exist far into the storage period.

The changes in total nitrogen (fig. 9) in the different varieties differ somewhat, but the results are too irregular to be of much significance.

The changes in acidity are also somewhat greater in certain varieties than in others as is evident from a comparison of Table Queen and King of the Mammoths (fig. 10).

The differences between varieties in the changes in astringency are generally not very important, as is evident from a comparison of the

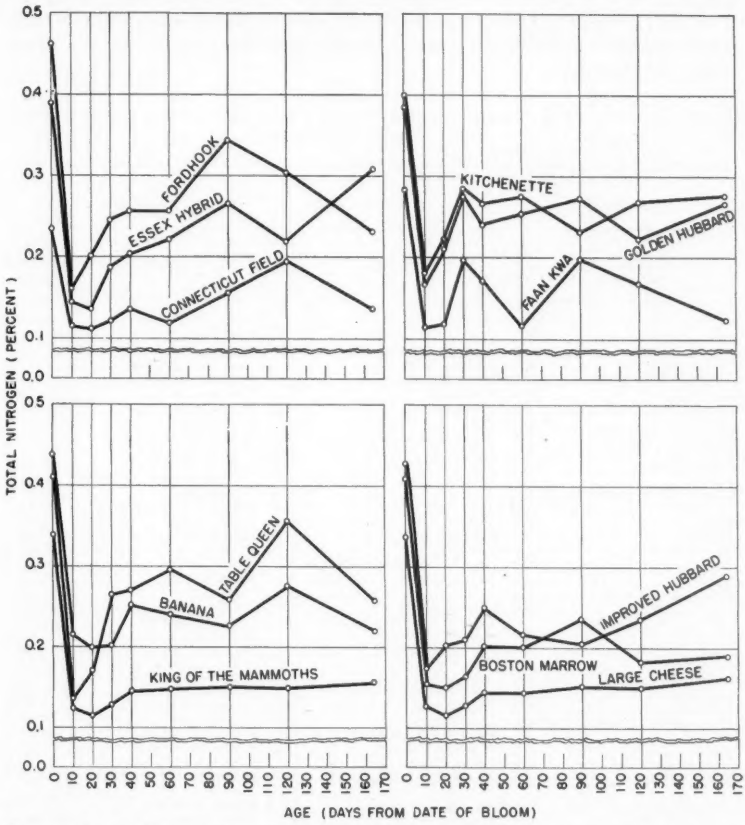

FIGURE 9.—Relation between the average total nitrogen content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

curves (fig. 11) for Fordhook and Connecticut Field, which show about the extreme differences.

COMPOSITION AND CULINARY USE

It is apparent from the reports of several workers (*6, 7, 8, 11, 13, 16*) that many differences in the taste and physical character of *Cucurbita* varieties and their adaptability to different uses are directly attributable to differences in their composition.

Numerous cooking and canning tests have been made with the material used in these analyses. Pumpkins are usually canned soon

after harvest or at about the stage of maturity of the material analyzed at 60 days of age. An examination of the material canned at this stage revealed wide differences in the color, flavor, and consistency of the canned products of different varieties. The ratings of the canned products (table 4) illustrate the way in which the varieties may differ. Some varieties are suited for one purpose and some for another; hence those considered best may differ greatly, depending upon the use to which they are to be put and the method of preparing them for this use.

TABLE 4.—*Rank of 36 Cucurbita varieties based on the color, flavor, and consistency of the canned product*

[Each value is an average for 3 years; the lowest value indicates the most desirable product in each case]

Color		Flavor		Consistency [1]	
Variety	Rating	Variety	Rating	Variety	Penetrometer reading
					Mm.
Boston Marrow	1.0	Golden Delicious	1.0	Delicious	17.2
Golden Delicious	1.3	Boston Marrow	1.0	Banana	21.2
Golden Hubbard	1.3	Golden Hubbard	1.3	Improved Hubbard	21.3
Essex Hybrid	1.5	Essex Hybrid	1.5	Blue Hubbard	21.3
Warren	2.5	Warren	1.5	Kitchenette	24.0
New England Pie	3.0	Delicious	1.5	Iron Bark	25.7
Winter Luxury	3.0	Improved Hubbard	2.3	Golden Delicious	25.8
Fort Berthold	3.0	Table Queen	2.3	Winnebago	26.0
Mammoth Golden Cushaw	3.0	Blue Hubbard	2.5	Golden Hubbard	26.1
Golden Oblong	3.3	Winnebago	3.0	Essex Hybrid	26.3
Gilmore	3.5	Chicago Warted Hubbard	3.0	Chicago Warted Hubbard	26.5
King of the Mammoths	3.5	Kitchenette	3.0	Gilmore	27.0
Omaha	4.0	Sibley	3.5	Warren	28.4
Delicious	4.0	Banana	3.5	Table Queen	28.6
Banana	4.5	Fordhook	3.7	Marblehead	28.7
Large Cheese	4.7	Gilmore	4.0	Sibley	28.8
Mammoth Chili	5.0	King of the Mammoths	4.5	Boston Marrow	31.1
Arikara	5.6	Mammoth Chili	4.5	Fordhook	31.2
Improved Hubbard	5.6	Arikara	4.6	Arikara	31.6
Blue Hubbard	6.0	New England Pie	5.0	Omaha	32.1
Kitchenette	6.0	Charles Naudin	5.2	Charles Naudin	32.3
Marblehead	6.0	Omaha	5.3	French Turban	32.5
Chicago Warted Hubbard	6.0	Golden Oblong	5.3	Tennessee Sweet Potato	33.2
Winnebago	6.5	Fort Berthold	5.5	Green Striped Cushaw	34.1
Table Queen	6.6	Winter Luxury	5.5	New England Pie	34.2
Faan Kwa	7.0	Mammoth Whale	5.5	Mammoth Whale	34.3
Fordhook	7.0	Marblehead	5.7	Golden Oblong	34.8
Japanese Pie	7.0	Iron Bark	6.5	Japanese Pie	35.7
Iron Bark	7.5	Connecticut Field	6.6	Fort Berthold	35.7
Sibley	7.5	French Turban	7.0	Winter Luxury	35.9
Connecticut Field	7.6	Large Cheese	7.0	Faan Kwa	36.5
French Turban	8.0	Mammoth Golden Cushaw	7.6	Mammoth Golden Cushaw	36.9
Charles Naudin	8.0	Faan Kwa	7.6	Mammoth Chili	37.9
Mammoth Whale	8.0	Japanese Pie	7.8	Large Cheese	39.4
Green Striped Cushaw	8.0	Green Striped Cushaw	8.0	Connecticut Field	40.9
Tennessee Sweet Potato	8.0	Tennessee Sweet Potato	8.2	King of the Mammoths	45.4

[1] Consistency was measured by means of a penetrometer originally devised for measuring the consistency of greases in accordance with specifications of the American Society for Testing Materials, for Test D 217–27 T (*1, pp. 421–426*). The lower the penetrometer reading, the thicker or heavier was the consistency of the product.

COLOR

The order of preference as to the color of the canned product was almost the same as that of the intensity of the yellow color of the carotenoid pigment. Sometimes there was a certain amount of darkening that detracted from the appearance of the material. Some varieties have green skins and the green color may extend into the flesh of the material; this gives the product a dull appearance. The

variety Delicious would have ranked higher had it not been for the dullness resulting from this green color. The color ranking of the varieties was nearly the same, no matter for what purpose the product was to be used. A bright, deep-yellow color was preferred in material to be boiled, cooked with butter or other seasoning, baked, fried, or made into pies. A few varieties with ratings between 1 and 1.5, such as Golden Delicious, Boston Marrow, and Golden Hubbard, were outstanding in appearance. Varieties ranking from

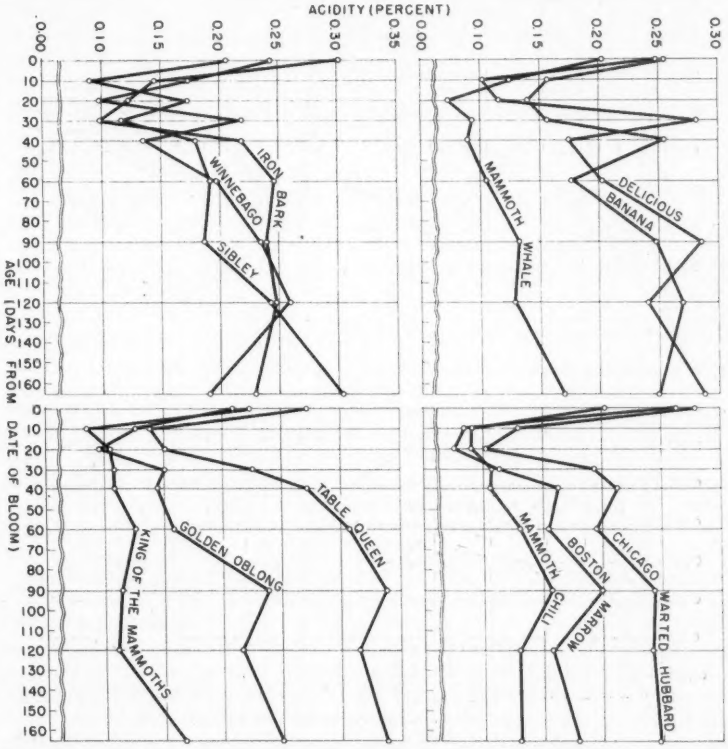

FIGURE 10.—Relation between the average acid content of the fruit of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

2.5 to 5.0, among which were New England Pie, Winter Luxury, Mammoth Golden Cushaw, and Large Cheese, were considered entirely satisfactory. Among the poorest were Mammoth Whale, Green Striped Cushaw, and Tennessee Sweet Potato, the first belonging to the *Cucurbita maxima* group and the last two to the *C. moschata* group.

FLAVOR

In contrast to the ratings for color, those for flavor varied greatly, depending upon the use to be made of the material and the preferences

of the individuals rating the varieties. In table 4 the order is based upon the flavor of the material just as it was emptied from the can. This order is practically the same as that based on the taste of the fresh material baked or steamed. The order of preference was considerably different when the material was made into pies. In many varieties the flavor of the canned or baked products was too strong

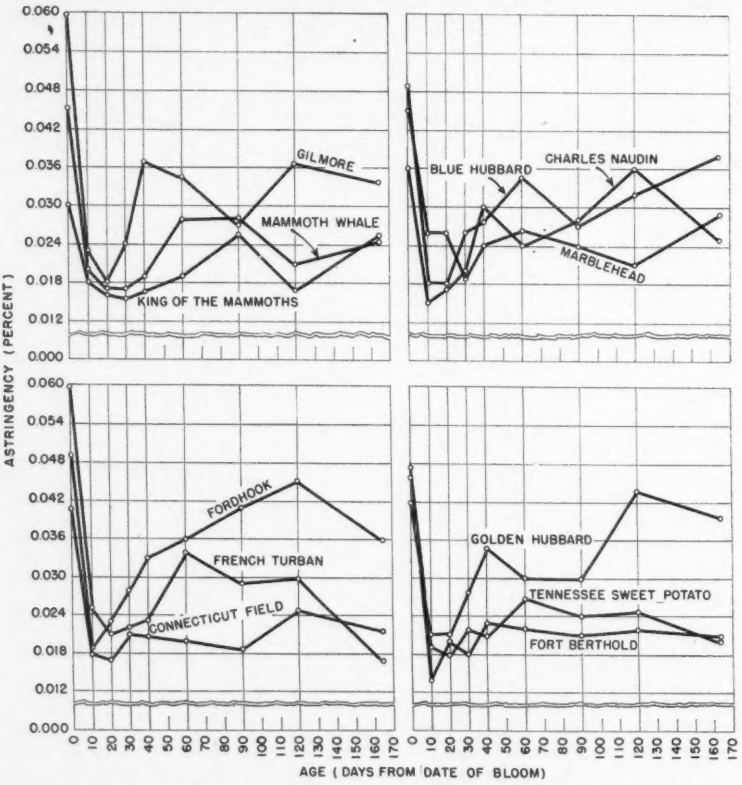

FIGURE 11.—Relation between the average total astringency content of the fruits of some *Cucurbita* varieties and their age, showing how the changes that occur differ in different varieties.

to be entirely pleasing. This was true particularly of varieties of the species *Cucurbita moschata*, such as Large Cheese and Mammoth Golden Cushaw. The flavor of these varieties was more agreeable, and hence they were rated higher in pies than when tasted directly, as in this case. The strong flavor of many varieties may account for the use of such spices as nutmeg and cinnamon in preparing table dishes of them. The varieties of the species *C. maxima* were generally much milder and were more often preferred than those of the other species. Some of the varieties of *C. pepo*, among which were Table Queen and Fordhook, were fairly mild in flavor, while others such as Winter Luxury and New England Pie, were rather strong.

The intensity of the characteristic pumpkin flavor varied greatly with the age of the material and the length of time that it remained in storage. The more mature the material or the longer in storage the stronger the flavor. The flavor of the immature material was mild, but it became much more intense between the 40- and 60-day stages and appeared to increase slowly during storage. The rapid increase in flavor in material from 30 to 60 days of age was accompanied by an increase in the brightness and apparent depth of the yellow color. It is during this period that ripening occurs.

Pleasing flavor apparently depends partly upon the sugar content. The rather high ranking of Table Queen, a *Cucurbita pepo* variety, seems to be due to the fact that it is exceptionally high in sugar. If sugar is added to Winter Luxury to make its sugar content equal to that of Table Queen, the differences in flavor between the two are greatly reduced.

Consistency

In the consistency measurements, the lowest reading indicates the heaviest, most viscous, or driest product. If the results in table 4 are compared with those in table 3, a reasonably good correlation will be found between acid-hydrolyzable polysaccharides and consistency, as well as between total solids and consistency. It is apparent that starch is more important than any other constituent in determining the consistency of the product. The cell-wall material and the pectin apparently do not vary widely and consequently do not cause great variations in the consistency, though they too are important.

Many varieties belonging to the species *Cucurbita maxima* were heavy in consistency, while a few were thin and watery; Delicious was at the top of the list and King of the Mammoths at the bottom. Table Queen and Fordhook, belonging to the *C. pepo* group, were moderately heavy, while the majority were comparatively thin. The species *C. moschata* contains no variety having a very heavy consistency.

The consistency of the product varied considerably with the age of the material. The very immature material, 10 to 20 days of age, had a very thin consistency when cooked or canned. Some varieties, like Delicious and Improved Hubbard, became much heavier in consistency from the 20- to the 40-day stage of maturity, while in other varieties this change was much less pronounced. Those varieties that increased greatly in total solids and acid-hydrolyzable polysaccharides increased correspondingly in heaviness of consistency. The consistency of the cooked products was heaviest at the 40- or 60-day stage of maturity. Those varieties with a heavy consistency at this stage generally became thinner in consistency during storage. The consistency of many varieties at the 40- and 60-day stages was considered too heavy for an ideal canned product. These varieties were greatly improved by storage for 30 to 60 days during which time much of the starch was changed to sugar.

When certain varieties, particularly those belonging to the species *Cucurbita maxima*, were canned by chopping the material into pieces ½ to 1 inch in diameter, packing them into cans, filling the interspaces with water, and then processing, the starch in the pieces cooked out into the water appeared white when cooled. The spaces between the pieces of material were solid or semisolid in character. This condition

made the product somewhat unattractive. It occurs sometimes in commercially canned pumpkin (*12*), in which case any space within the can not occupied by the pulped material becomes filled with the white starchy substance. These white spaces or patches have been called starch pockets or starch balls. There is a direct relation between the occurrence of these starch pockets, the consistency of the product, and the starch content of the material. Starch pockets are most likely to occur when the material is canned early in the season. In these tests it sometimes occurred even in such varieties as Large Cheese and New England Pie when canned very early. Starch pockets have been noted in Table Queen canned at 40 days of age but not in material that had been stored. Where this condition arises, it can almost always be improved by storing the pumpkins for 60 days or more. It may not occur even in varieties such as Delicious and Improved Hubbard after they have been stored for 60 days or more.

Palatability of Immature Materials

Most of the varieties may be used when immature, as is the custom with summer squash. At 20 to 30 days of age the fruits of some varieties of *Cucurbita maxima* might well be considered delicious. The varieties Tennessee Sweet Potato and Japanese Pie sometimes had an objectionable bitterness that made them less acceptable. Varieties with a low starch content appeared to have too much water or to lack substance. King of the Mammoths, Mammoth Chili, and Connecticut Field were varieties of this character. Delicious, Improved Hubbard, and Golden Hubbard were exceptionally good at 20 to 30 days of age.

Nitrates and the Corrosion of Cans

A study of the canned material revealed a considerable difference among varieties in their action on the tin containers. Table Queen corroded cans very little, while Chicago Warted Hubbard often corroded them severely. Generally when nitrates were low or absent the cans showed little corrosion, but when they were high the action on the cans was very severe. Sometimes young material caused severe corrosion, whereas material stored for 30 to 60 days caused much less. Of course nitrates were not the only constituents favoring the corrosion of cans; oxygen in the container was also important.

Discoloration

In these tests there was very little tendency for the material to darken during ordinary cooking or during canning. Many varieties were practically free of any objectionable darkening. This was probably due to the very low content of astringency or hydroxy-aromatic substances that react with iron. In some of the products a slightly dull appearance was noted, obviously due to the presence of chlorophyll in the fresh material.

SUMMARY

Thirty-six varieties of pumpkins and winter squashes, grown for 1 to 4 years, were analyzed at different stages of development and

after different periods of storage. Statistical analyses of the data indicated rather large differences in the extent to which many of the constituents varied, the sources of greatest variation being differences in age of the material and differences among varieties.

When calculated as percentage of the fresh weight the mean total-solids content decreased during the first 10 days of growth and then increased to the 40-day stage, after which there was a slow decline. The sugars increased during the developmental period and the first 60 days of storage. The acid-hydrolyzable polysaccharides increased rapidly to the 30- or 40-day stage and then almost as quickly decreased to the 90-day stage, after which the decline was slow. Although the changes were not very important, the acidity, astringency, and total nitrogen content decreased to the 10- or 20-day stage and then increased slowly to the end of the storage period. The nitrate nitrogen increased to the 20-day stage and then declined to the end of the storage period, the direction of the changes being the reverse of those in the total nitrogen.

When the results for each constituent for all stages of maturity in all years were averaged it was found that in general the varieties markedly high in sugar were also markedly high in acid-hydrolyzable polysaccharides; that those high in total solids were high in soluble solids and only moderately high in total nitrogen.

The varieties differed greatly, however, in the extent of the changes that occurred in the content of total solids, acid-hydrolyzable polysaccharides, and sugars. The differences in the acidity, astringency, and total nitrogen content of the varieties were of much less importance. There were comparatively small differences in most constituents of the fruits during the very early stages of development, but the differences often became pronounced as the fruit approached maturity. The varieties that were high in acid-hydrolyzable polysaccharides at the time of maturity generally became high in sugar during storage as a result of the conversion of starch into sugar. Among those varieties high in acid-hydrolyzable polysaccharides or sugar were Table Queen, Fordhook, Banana, Improved Hubbard, Golden Hubbard, and Sibley. Among those low in these constituents were Connecticut Field, King of the Mammoths, Large Cheese, Mammoth Whale, and Winter Luxury. *Cucurbita pepo* and *C. maxima* both contained some varieties that were high and others that were low in dry matter and polysaccharides, but *C. moschata* contained no varieties that were exceptionally high in these constituents. In a few varieties, such as Table Queen and Fordhook, nitrate nitrogen was almost absent after the fruits reached maximum size, whereas in other varieties, such as Marblehead and Kitchenette, appreciable amounts were present far into the storage period.

Differences in the flavor, consistency, and appearance of *Cucurbita* varieties were often very great and were directly related to differences in composition. Because of these differences some varieties that are excellent for one purpose may be only mediocre for another. The period when *Cucurbita* fruits have maximum value depends upon the variety and the particular use to be made of the fruits. Some varieties are excellent for certain culinary uses during the early stages of maturity and very poor for the same uses at later stages, while the reverse may be true of other varieties.

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